31ST ISTA CONGRESS
Tallinn | Estonia 2016

ISTA Seed Symposium
Abstracts

31st ISTA Congress
Tallinn, Estonia
15–17 June 2016

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Preface

Dear Seed Symposium Participants

Welcome to Tallinn, and to the 31st ISTA Seed Congress.

The theme of this years' Seed Symposium is Progress in seed testing and seed quality improvement through science and technology. This theme was chosen to reflect the fact that Estonia is well-known for making progress by technological advancement, a theme that we are always keen to reflect in the world of seed testing.

The Symposium is made up of five sessions, each with a lead speaker well known in their field. There will be a total of 30 oral presentations and 87 poster presentations, the abstracts of each of the presentations are included in this book. I am very pleased that Session 4 is a collaborative session with the International Society for Seed Science. Bringing together those involved in fundamental aspects of seed science with those who work in seed testing is key for the development of seed analysts and seed testing, and this is seen as very important to ISTA.

The ISTA Seed Symposium brings together seed scientists, seed technologists and seed analysts working in seed testing laboratories, research departments and private companies. It represents a unique opportunity to hear about the most up to date research in seed science and developments in seed testing, as well as a chance to meet and network with those involved in seed research and seed testing around the world. Whether this is your first experience of an ISTA Seed Symposium, or you have attended many Symposia over the years, I would encourage you to take the opportunity to not only enjoy the great programme of presentations and posters that we have put together for you, but also to take advantage of this opportunity to meet with seed testing colleagues, to exchange ideas and to plan future collaborations – meetings such as this allow seed testing to remain up to date with current research and technology and progress even further.

I very much hope that you enjoy the Seed Symposium, and that you are able to fully take advantage of the opportunities that this meeting provides you with.

Laura Bowden
ISTA Seed Symposium Convenor

Contents

PROGRAMME 6

ORAL PRESENTATIONS 11

ORAL SESSION 1 – APPLICATIONS OF GERMINATION AND DORMANCY TESTING 11
Fernando Silveira *(Keynote speaker)*

ORAL SESSION 2 – ADVANCES IN SEED HEALTH TESTING FOR ENSURING QUALITY DURING SEED PRODUCTION AND STORAGE 16
Gary Munkvold *(Keynote speaker)*

ORAL SESSION 3 – Environmental Effects on Seed Quality 21
Ilse Kranner *(Keynote speaker)*

ORAL SESSION 4 – ISSS COLLABORATIVE SESSION – CELLULAR AND MOLECULAR METHODS AND NEW APPROACHES TO SEED QUALITY DETERMINATION 25
Julia Buitink *(Keynote speaker)*

ORAL SESSION 5 – CONSERVATION AND USE OF GENETIC REOURCES IN CROP, FOREST AND WILD SPECIES 29
Mati Koppel *(Keynote speaker)*

POSTER SESSIONS 34

POSTER SESSION 1 – APPLICATIONS OF GERMINATION AND DORMANCY TESTING 34

POSTER SESSION 2 – ADVANCES IN SEED HEALTH TESTING FOR ENSURING QUALITY DURING SEED PRODUCTION AND STORAGE 49

POSTER SESSION 3 – ENVIRONMENTAL EFFECTS ON SEED QUALITY 57

POSTER SESSION 4 – ISSS COLLABORATIVE SESSION – CELLULAR AND MOLECULAR METHODS AND NOVEL TECHNIQUES FOR SEED QUALITY EVALUATION 66

POSTER SESSION 5 – CONSERVATION AND USE OF GENETIC REOURCES FOR CROP, FOREST AND WILD SPECIES 77

Programme

Wednesday, 15 June 2016

08:00–17:00	Congress Registration desk open	
08:30–17:00	ISTA Seed Symposium day 1	
08:30–09:30	Opening ceremony	
	▪ Welcome by the Minister of Rural Affairs of the Republic of Estonia, Urmas Kruuse	
	▪ Deputy Chair of the National Organising Committee (NOC), Sigmar Suu	
	▪ ISTA President, Joël Léchappé, France	
09:30–10:00	Presentation: The Seed Industry in Estonia, Estonian Seed Association, Manager, Christel Mölder	
10:00–10:30	Coffee break	
10:30–11:00	**ISTA Seed Symposium 2016: Progress in seed testing and seed quality improvement through science and technology.** **Symposium Convenor:** Laura Bowden, Science and Advice for Scottish Agriculture, UK	
11:00–12:30	**Session 1: Applications of germination and dormancy testing** Fernando Silveira, Federal University of Minas Gerais, Brazil **Using tools from ecology and evolution to maximise the use of native seeds in species conservation and habitat restoration**	
	Digital imaging of seed germination	Didier Demilly, Sylvie Ducournau, Marie-Hélène Wagner, Carolyne Dürr GEVES, France
	Testing for viable seeds in Grain Screening Pellets	Steve Jones, Nicole Wurm, Ruojing Wang, Sharon Leung CFIA, Canada
	Origin of variation in spinach germination results between laboratories	Lucile Daron, Meindert Klooster, Rafael Chan-Navarrete, Anton Grim ENZA ZADEN, The Netherlands
12:30–13:30	Lunch break	

13:30–14:30	**Session 1 (continued)**	
	Application of the radicle emergence test to assess seed vigour of cereal and forage crops	Mohammed Khajeh-Hosseini, Rahmatolah Gheshm, Mostafa Cheshmi Ferdowsi University of Mashhad, Iran
	Germination performance of damaged maize seeds evaluated by micro-computed tomography and image processing	Francisco Gomes-Junior, Silvio Cicero, Carlos Vaz, Lúcio Jorge University of São Paulo, Brazil
	On-line proficiency tests elucidate the cause of under-dispersion in germination testing	Michael Kruse, Peter Deplewski University of Hohenheim, Germany
14:30–15:00	**Poster session 1**	
15:00–15:30	Coffee break	
15:30–17:00	**Poster session 1 (continued)**	
17:00-20:00	Time allocated for ISTA TCOMs side meetings	

Thursday, 16 June 2016

08:00–16:30	Congress Registration desk open	
08:30–16:30	ISTA Seed Symposium day 2	
08:30–10:00	**Session 2: Advances in seed health testing for ensuring quality seed production and storage** Gary Munkvold, Iowa State University, USA **Emerging disease issues affecting international seed movement and seed health test development**	
	Storage of cereal seeds may reduce *Fusarium/Microdochium* infection frequencies and increase germination	Guro Brodal, Margit Kim, Birgitte Henriksen, Håkon Tangerås Nibio, Norway
	TESTA project: Transmission from seed to plant and from soil to plant of *Tilletia* caries (early detection by PCR and damage threshold)	Geoffrey Orgeur, Audrey Chamaille, Isabelle Serandat, Aurélie Dupuy, Matthieu Rolland GEVES, France
	Effect of biopriming seed treatment with Rhizobacteria on plant growth of hot pepper (*Capsicum annuum* L.) and incidence of *Phytophthora* blight	Satriyas Ilyas, Aulia Zakia, Candra Budiman, Dyah Manoharah Bogor Agricultural University, Indonesia
10:00–10:30	Coffee break	

10:30–11:30	**Session 2 (continued)**	
	Development of quantitative and qualitative test methods for detection of wheat bunt diseases (*Tilletia spp.*) by means of Real-Time PCR assays	Monika Grundler, Monika Sedlmeier, Benno Voit, Berta Killermann, Ludwig Niessen Bavarian State Research Center for Agriculture, Germany
	Integrating the effects of sampling and analysis to produce sampling plans for seed-borne pests and pathogens	Valerie Cockerell, Roy Macarthur, Jane Thomas, Mauro Dal Pra, Maaike Bruinsma, Harrie Koenraadt, Ilaria Alberti SASA, UK
	Umbel browning and stem necrosis in carrot seed production in France: isolation and characterization of the fungal pathogen	Frank Bastide, Isabelle Serandat, Pascal Poupard, Philippe Simoneau, Valérie Grimault GEVES, France
11:30–12:30	**Poster session 2**	
12:30–13:30	Lunch break	
13:30–14:30	**Poster session 2 (continued)**	
14:30–15:00	**Session 3: Environmental effects on seed quality** Ilse Kranner, Univeristy of Innsbruck, Austria **Effects of the maternal environment on seed quality**	
15:00–15:30	Coffee break	
15:30–16:30	**Session 3 (continued)**	
	The effect of environmental conditions during seed production on seed development and vigour	Birte Boelt, Dot Vittrup Pedersen, Karsten Hartelius, Jens Michael Carstensen Aarhus University, Denmark
	Effect of simulated rainfall at different stages of wheat seed development and maturation on subsequent seed longevity	Richard Ellis, Gajender Yadav University of Reading, UK
	Comparison of the effects of high temperatures during grain filling on grain composition and seed quality of wheat, oilseed rape and pea	Carolyne Dürr, Sophie Brunel-Muguet, Annabelle Larmure, Christine Girousse, Agnès Sabaté, Colette Larré, Marie-Hélène Wagner INRA, France
17:00–19:00	Time allocated for ISTA TCOMs side meetings	

Friday, 17 June 2016

08:00–17:00	Congress Registration desk open	
08:30–17:00	ISTA Seed Symposium day 3	
08:30–09:30	**Session 3 (continued)**	
	Influence of seed production environment for oilseed rape germination and vigour	Simon Goertz, Marie-Hélène Wagner, Amine Abbadi, Sylvie Ducournau, Zelkjo Micic, F Breuer, D Dugue, G Leckband NPZ, Germany
	Environmental and other factors affecting the quality of wheat seed produced in Scotland	Laura Bowden, Adrian Roberts, Valerie Cockerell SASA, UK
09:30–10:00	**Session 4: (ISSS Collaborative Session) Cellular and molecular methods and new approaches to seed quality determination** Julia Buitink, INRA, France **A systems biology approach to understand and predict seed quality**	
10:00–10:30	Coffee break	
10:30–12:30	**Session 4 (continued)**	
	Discovery of seed longevity genes through genome-wide association mapping using high-density SNP array	Jaesung Lee, Dmytro Chebotarov, Kenneth McNally, Fiona Hay IRRI, Philippines
	A new spectral imaging based technology for phenotyping and classification of seed samples	Karsten Hartelius, Henning van Veldhuizen, Kim Nielsen, Michael Carstensen Videometer A/S, Denmark
	A new approach to prediction of normal germination and vigour based on rate of radicle emergence	Alison Powell, Linda Kerr, Marie-Hélène Wagner, Kazim Mavi, Stan Matthews University of Aberdeen, UK
	The Involvement of Enclosing Maternal Tissue Layers in Seed Dormancy	Robert Geneve, Sharon Kester University of Kentucky, USA
	Effects of Seed Priming with Sodium Nitroprusside and Trehalose on Improvement of Chilling and Drought Tolerance of Tobacco Seeds and Seedlings in Relation to Proline Metabolism	Zheng-Ling Long, Wen-guang Ma, Sha-sha Wang Ming Gong Yunnan Normal University, China
	Analyses of several seed viability markers in individual recalcitrant seeds of *Eugenia stipitata* McVaugh with totipotent germination	Geângelo Calvi, Fabiana Aud, Isolde Ferraz, Hugh Pritchard, Ilse Kranner National Institute for Amazonian Research, Brazil
12:30–13:30	Lunch break	

13:30–15:00	**Session 5: Conservation and use of genetic resources in crop, forest and wild species.** Mati Koppel, Estonian Crop Research Institute, Estonia	
	Conservation and use of genetic resources in crop, forest and wild species in Estonia	
	Seed storage in *ex situ* genebanks - inter- and intraspecific variation and genetic determination	Andreas Börner, Mian Abdur Rehman Arif, Mai Allam, Monika Agacka-Moldoch, Ulrike Lohwasser, Mariann Börner, Manuela Nagel IPK, Germany
	Practical aspects of testing seed moisture using equilibrium relative humidity (eRH)	Robert Karrfalt USDA Forest Service, USA
	Genebank seed accession phenotyping through spectral imaging	Jens Michael Carstensen, Karsten Hartelius, Fiona Hay Videometer A/S, Germany
15:00–15:30	Coffee break	
15:30–16:30	**Session 5 (continued)**	
	How to germinate seeds of bitter gourd (*Momordica charantia L.*) after storage at subzero temperatures	Sebastian Bopper, Maximiliane Wacker, Michael Kruse University of Hohenheim, Germany
	Perspectives on the conservation and restoration of native wild legumes: the biology and ecology of *Astragalus, Oxytropis* and *Lathyrus* species as models.	Erica Dello Jacovo, Euan James, Gregory Kenicer, Tracy Valentine, Costantino Bonomi and Pietro Iannetta The James Hutton Institute, UK
	Water content effect on seed longevity	Sara Mira, Elena Estrelles, Maria Elena Gonzalez-Benito University of Madrid
16:30–17:00	**Overview of symposium** Conclusion of Symposium	Andreas Börner IPK, Germany Laura Bowden, Science and Advice for Scottish Agriculture, UK
17:00	**Official Photo**	
19:30–23:30	Official Congress Dinner and Presentation of Seed Symposium Awards	

Oral Presentations

ORAL SESSION 1 – APPLICATIONS OF GERMINATION AND DORMANCY TESTING

Keynote presentation: Using tools from ecology and evolution to maximise the use of native seeds in species conservation and habitat restoration

Fernando A. O. Silveira[1], Daniel Negreiros[2] and Roberta L. C. Dayrell[1]
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Unsustainable human population growth has resulted in pervasive and unparalleled levels of habitat destruction. Direct consequences of habitat loss are leading to worldwide demands for knowledge on seed biology that can be directly applied to species conservation and landscape-level habitat restoration. However, there are several knowledge gaps in the seed biology of native species because the factors that control their seed development, germination and dormancy are drastically different from those of crop species. The underlying cause of these differences is that seed traits of crops were shaped by artificial selection whereas those of native species were shaped by natural selection. Despite the growing interest in the ecological and evolutionary factors that drive seed biology of native species in recent decades, the rate of habitat destruction is much higher than our ability to generate scientific data on native species seed biology, and thus our capacity to effectively restore degraded environments is severely limited. Here, we use tools from evolutionary biology in order to improve our ability to predict germination behaviour of species that have never been studied before. Specifically, we implement analysis of phylogenetic signal, (a trend for related species to resemble each other more than species drawn at random from a phylogenetic tree - a diagram showing species relationships based on common ancestry), and phylogenetic independent contrasts (correcting for data dependency in regression analysis). As an example, we use a germination database of species from *campo rupestre*, a megadiverse, nutrient-limited grassland of eastern Brazil in which seed quality issues are recurrent. First, we provide a theoretical framework for assessing the quality of wild seeds which are markedly different from crop species. Then, we discuss how analyses of phylogenetic signal and phylogenetic independent contrasts work and show their relevance to increase our predictive power on germination patterns across neglected clades. We conclude by arguing that 1) in order to fully understand the challenges for managing native species one should understand basic ecological and evolutionary principles that drive their germination and 2) bridging the gap between seed technologists and seed ecologists will benefit both areas and enhance our ability to restore degraded ecosystems following human disturbance.

Digital imaging of seed germination

Didier Demilly[1], Sylvie Ducournau[1], Marie Hélène Wagner[1] and Carolyne Dürr[2]
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Germination *sensu stricto* begins with the uptake of water by the seed and terminates with the initiation of growth of the embryo axis and radicle protrusion. Cumulative results from individuals provide germination time curves. This trait is often used to evaluate the physiological quality of seeds. It is easier to detect the radicle protrusion than to estimate the seedlings and ungerminated seeds in a germination test. Nevertheless, in optimal conditions, time of radicle protrusion detection requires periodic observations even during the night and weekends for good reliability. Such constraint prevents the use of this test. Automated image analysis overcomes these manual periodic counts. Since 2000 we have developed such automatic analysis. Our experience has increased, according to species (more than 50), throughput (up to 7000 seeds/run, 80 000 seeds /year from 15 000 images), environmental conditions monitoring and applications. Individual results allow precise correlation of single seed germination with subsequent traits such as dry seed weight and size, imbibition and growth. These data complement those of the germination test. Improvements in equipment, sowing tools, imaging and analysis of images and data facilitate such analysis. The package Visigerm is an ImageJ/Fiji set of macros available on website www.geves.fr. It allows the obtaining of a germination time curve from a digital image sequence of seed germination. Recent studies relate to the variability of germination among species and the usefulness of such a test for longevity testing.

Testing for viable seeds in Grain Screening Pellets

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Grain Screening Pellets (GSP) are sold as animal feed in North America. Because the material in the pellets comes from grain screenings there are weed and crop seeds present in the pellets. During processing the screened material is ground, pelleted and heat treated. To ensure this product complies with regulatory requirements, and is not a route for noxious/regulated weed seeds to move between countries, there is the need to ensure any intact seeds in the pellets are non-viable. The CFIA laboratory in Saskatoon, in collaboration with the USDA (Beltsville, MD), developed a test that combined different elements of ISTA and AOSA testing procedures to detect any viable seeds in the GSP product. A negative (no viable seeds found) test result, combined with on-site inspection of GSP processing facilities, is now used to approve facilities to produce the GSP product in Canada for export to the USA. Working sample and testing methodology were based on ISTA or AOSA test method principles. Seed extraction, retrieval and identification procedures were combined with a modified ISTA/AOSA tetrazolium test to determine viability of any intact seeds found in the pellets. Viable crop seeds are used as a control for the viability testing. The method was validated by exchanging GSP test samples between the official testing laboratories in Canada and the USA. Data is presented on the species of seeds that remain intact during GSP processing and the incidence of viable seeds found.

Origin of variation in spinach germination results between laboratories

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Results from spinach (*Spinacia oleracea* L.) germination tests are often significantly different when performed by several laboratories. The high variability of these results can create conflicts between seed companies, producers and reference institutes. Often, a poor correlation exists between laboratory results and field performances. In spinach, an undeveloped rooting system is believed to cause poor field performances. Thus, 8 seed companies started a project in 2011, in cooperation with the Naktuinbouw, to identify the origin of the variation in spinach germination results, with attention to the rooting system. The root characteristics and plants phenotypes were correlated and standard root criteria in laboratory germination tests were used to decrease the results variability. We found a strong positive correlation between root length (RL) and Fresh Weight (FW) and the majority of plants with a growth delay had a 'short' root (Root:Hypocotyl <1). Three ring tests were organized: the first one (RT1) with free germination method and assessment criteria, RT2 with standardized germination method (PP10, 14DAS) but free criteria and RT3 with both standardized method and criteria. There was a high variability in germination results for RT1 and RT2, which was significantly decreased in RT3. We proved that standardized root criteria during spinach germination tests could increase results uniformity between laboratories. Creating a separate 'short root' quality category would improve the reliability of laboratory tests to predict field emergence and meet seed companies' needs.

Application of the radicle emergence test to assess seed vigour of cereal and forage crops

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Despite the vast cultivated area worldwide of cereal and forage crops such as wheat and alfalfa and their importance in human and animal nutrition their seed vigour testing has not been standardized. The radicle emergence (RE) test has however been applied to maize and may therefore have potential in wheat and alfalfa. Early counts of germination (radicle emergence) were taken at 6 hourly intervals at temperatures of 20, 17, 15 and 13 °C for 18 seed lots of wheat and 14 seed lots of alfalfa, all having standard germination above 85%. Mean germination time (MGT) was also calculated. Field emergence was carried out for both species on 4 replications of 50 seeds from each seed lot in a complete block design and emerged seedlings counted daily to assess mean emergence time (MET) and final emergence. Cell cycle activation and DNA replication during imbibition and germination at a range of temperatures was also studied using flow cytometry for lots showing a range of vigour. In general, in both species as temperature decreased, the rate of germination reflected in MGT and the final germination decreased. The effects of temperature were greater on the germination rate than on the final germination percentage. Many of the early counts of radicle emergence

at all germination temperatures were correlated with MET and final emergence in both wheat and alfalfa. However at lower temperatures radicle emergence took place later. RE vigour tests after 48 hours at 15 °C and 24 hours at 20 °C, are proposed as the most convenient timing and temperature for wheat and alfalfa respectively. The relationship between RE and the cell cycle is discussed. Cell cycle activation is proposed as a possible molecular marker for seed vigour testing.

Germination performance of damaged maize seeds evaluated by micro-computed tomography and image processing

Francisco Guilhien Gomes-Junior, Silvio M. Cicero, Carlos M. P. Vaz and Lúcio A. C. Jorge
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Seeds with different levels of mechanical damage sorted by digital X-ray were analyzed using a SkyScan 1172 benchtop micro-computed tomography system (SkyScan, Belgium). The reconstructed sections of each seed were analyzed by DataViewer software (version 1.5.1.2). The 2D transaxial, coronal and sagittal sections were used to inspect the seed anatomy and changes caused by mechanical injuries in internal tissues. ImageJ software was applied for image processing and determination of damaged area in two transaxial sections (T1- endosperm and T2- endosperm + embryo) compared to the total area of each section. Germination tests were performed using rolled paper towels (25 °C for 7 days) and seedling length was evaluated daily. Seedling dry mass was also assessed after seven days of germination. Micro-computed tomography produced high-resolution images (6.5 µm) and allowed better mechanical damage characterization compared to X-ray images. Damaged areas ranged from 0.2 to 56.7% and 0.4 to 20.4% for T1 and T2, respectively. After two and three days of germination, higher seedling length occurred for most of damaged seeds (without injury in the embryonic axis) compared to intact seeds; however, the seedling length and seedling dry mass from those damaged seeds was lower at the seventh day of germination.

On-line proficiency tests elucidate the cause of under-dispersion in germination testing

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Evaluations of germination test data continually report under-dispersion, i.e. the four replicate results vary less than to be expected in a random experiment. To elucidate the cause we organized two on-line proficiency tests with together 6 samples each containing 4 replicates of 100 computer generated images of cereal-like seedlings. All participants classified the same images as either normal or abnormal according to given thresholds for shoot-root ratio. The empirical classifications were compared with the known true identity of the seedlings.

Significant under-dispersion was found in 5 out of the 6 samples. Mean rates of falsely classified normal and falsely classified abnormal seedlings were 19.6% and 3.5%, respectively. Computer simulations showed that random occurrence of errors with these rates is not causing under-dispersion but the over-proportional occurrence of false normal seedlings in replicates with low normal percentages and the over-proportional occurrence of false abnormal seedlings in replicates with high normal percentages. The results of already completed replicates did not always affect decision making in the coming replicates. Thus, obviously, decision making about seedlings is subliminally affected by assumptions, expectations or habits of the analyst about the variation among replicates. In cognitive psychology this is called “confirmation bias”. Combining of replicate results from different analysts decreased under-dispersion significantly and also over-dispersion among tests. So the confirmation bias is not constant among analysts.

ORAL SESSION 2 – ADVANCES IN SEED HEALTH TESTING FOR ENSURING QUALITY DURING SEED PRODUCTION AND STORAGE

Keynote Presentation: Emerging disease issues affecting international seed movement and seed health test development

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International movement of seed is a critical component of modern agriculture; the volume and value of seed shipments continues to increase year by year. Seed is recognized as a pathway for the movement of plant pathogens, and it is in the best interest of all stakeholders to take measures that will minimize this risk. Seed health testing is a crucial tool for risk management, but it can be expensive and time-consuming. Therefore it is important to understand under what circumstances it is appropriate to require seed health tests. This is challenging due to the dynamic nature of international trade, pathogen populations, and phytosanitary policies. These changing factors lead to a continuous need for new research and development in seed health testing methods. Several recent examples will be discussed that illustrate the challenges of maintaining safe international seed movement and defining appropriate seed health testing methods. Maize lethal necrosis recently emerged as a serious threat to maize productivity in eastern Africa. This disease is caused by a combination of two viruses, and the recent outbreak is linked to the appearance of *Maize chlorotic mottle virus* (MCMV) in the region. The virus can be seed-transmitted but there is a poor correlation between incidence of seed infection and the risk of seed transmission. MCMV populations are diverse, and current detection methods may not be sufficiently specific and sensitive. *Cucumber green mottle mosaic virus* (CGMMV) was recently introduced into vegetable production areas in California, USA. Both of these examples have led to changes in phytosanitary requirements and an acute need to re-evaluate existing seed health testing methods. Pospiviroids have become an increasing concern in the movement of tomatoes and other Solanaceous crops. *Potato spindle tuber viroid* (PSTVd) and its relatives can be seed-transmitted, but like MCMV, the relationship between seed infection and transmission is unpredictable. Viroid detection methods are limited, and phytosanitary requirements differ widely among countries. In all three of these cases, methods are needed that will distinguish between infectious and non-infectious or inactivated virus particles or RNA. Two other examples, *Phomopsis* spp. on spinach seed, and *Pepper mild mottle virus* (PMMoV), will be discussed, illustrating the need for international cooperation and standardization of seed health testing methods.

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Storage of cereal seeds may reduce *Fusarium/Microdochium* infection frequencies and increase germination

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Fusarium and *Microdochium* may cause seedling blight and poor germination of cereal seeds. However, indications of poor survival of *Fusarium* in seed and improved germination after some months of storage have been observed. A study was carried out to investigate if seed storage can contribute to improved seed quality. Samples from seed lots of barley, oats and spring wheat were tested for germination capacity and *Fusarium*/*Microdochium* infection frequencies a few days after harvest, and after 5, 12 and 15 months of storage. In barley, the average germination percentage increased slightly, from 92% at harvest to 95% after five months of storage. In oats, the average germination percentage increased from 82% to 85% during the first five months. In spring wheat, the average germination percentage was reduced from 81% at harvest to 67% after five months. In barley and oats, average *Fusarium*/*Microdochium* frequencies were reduced during storage, with the highest reduction observed during the first five months (from 50% to 37%, and from 60% to 46%, barley and oats respectively). In spring wheat, no significant reduction in average infection level was recorded (58% at harvest, 50% after 15 months of storage). There was however, variation between seed lots in all three cereal species in both germination percentage and *Fusarium*/*Microdochium* frequencies during the storage period. It is concluded that storage of barley and oats seeds for 5 months after harvest may in some cases increase the seed quality and thereby meet the certification requirements of minimum 85% germination.

Transmission from seed to plant and from soil to plant of *Tilletia caries* (early detection by PCR and damage threshold)

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A study funded by the TESTA project aimed at defining a protocol to study the damage threshold and transmission of *T. caries* from seed to plantlet and from soil to plant. Current protocols to study transmission of *T. caries* use naturally or artificially contaminated seeds sown in the field and notation of bunted ears, which are long and time consuming. The aim of our study was to develop a protocol which was not dependant of environmental conditions, not time consuming and could give an early result. Artificial seed and soil contamination (adapted from CEB method N°42) with different contamination rates of viable spores and non-viable spores were done to evaluate the threshold damage of *T. caries*. The assays were conducted in climatic chamber according to Eibel *et al*. in 2005 and a PCR test was developed at an early plant stage. For the plants transferred in field and greenhouse, results showed that a low contamination rate of seeds by *T. caries*, was able to induce symptoms in ears the next year

and confirmed the capacity of transmission of *Tilletia caries* in low concentration. In comparison with the symptom expression observed, the results showed that the early detection by PCR was better in stem six weeks after sowing. Correlation between PCR detection and symptom expression will be presented to show the efficiency of the protocol to study transmission of *T. caries* from seed to plantlets, from soil to plant and its usefulness to access efficiency of seed treatments, in a faster way than the actual protocol in field. Recent development of a qPCR protocol will be presented.

Effect of biopriming seed treatment with rhizobacteria on plant growth of hot pepper (*Capsicum annuum* L.) and incidence of *Phytophthora* blight

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Phytophthora capsici is a seed- and soil-borne fungal pathogen causing phytophthora blight in hot pepper. The disease is difficult to control due to the unavailability of resistant varieties in Indonesia. The purpose of this research was to isolate rhizobacteria that can inhibit *P. capsici*, and to evaluate the effect of seed treatments with rhizobacteria in enhancing plant growth in a green house. Exploration of rhizobacteria was conducted at the production central of hot pepper in East and West Java. Rhizobacteria were isolated from the rhizosphere or rhizoplane of healthy plants among the ones with symptoms of phytophthora blight. A rhizobacteria inhibition test against *P. capsici* was conducted using dual culture method. Selected rhizobacteria isolates were further tested for their compatibility using dual cultures. Then selected isolates were used for biopriming seed treatment on hot pepper seeds cv. Laris, which is susceptible to phytophthora blight. Seeds were soaked in a suspension of rhizobacteria isolate(s) 108 cfu for 24 h at 20°C. The suspension was made by incubating the rhizobacteria cells in 50 ml potato dextrose for 48 h. Effects of biopriming were evaluated on seedling/ plant growth. Twenty two of 252 isolates showed their potential to inhibit *P. capsici*. The highest inhibition was shown by E1 isolate (58%), a combination of E1+E3C2 isolates (58%), E1+F2B1 (60%), and E1+E3C2 +F2B1 (58%). Biopriming with a combination of E1+F2B1 isolates resulted in the best seedling growth up to 42 days after sowing in the seedbed, and better growth 2 weeks after transplanting (wat) in polybags. Soil inoculated with *P. capsici* was spread around the base stem at 4 wat. The effects of biopriming on plant growth and incidence of phytophthora blight were also evaluated.

Development of quantitative and qualitative test methods for detection of wheat bunt diseases (*Tilletia* spp.) by means of Real-Time PCR assays

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In organic seed multiplication as well as for seed trading reliable and effective testing methods for the determination of teliospores of *Tilletia* spp. on wheat are very important. Internationally quarantine regulations and different thresholds must be considered. At present the determination of the teliospores is carried out according to the ISTA Working Sheet No 53 which is a microscopy method requiring a lot of experience to distinguish the different *Tilletia* species. The aim of the research project started in January 2015 is to develop a selective, sensitive, robust and reproducible molecular testing method by means of quantitative Real-time PCR (qPCR) to facilitate the qualitative and quantitative determination of different *Tilletia* species. Teliospore samples from different areas of Germany, neighboring countries and the USA (*Tilletia laevis*) are used at present for genetic analyses. Bunt balls from infested ears provide species specific spore material. After being morphologically verified the genomic DNA will be isolated by commercially available kits. Different microsatellite markers and primers for genes known from phylogenetic analyses and related fungi are used for PCR to detect relevant gene regions useable for the development of species specific primers and probes. The results achieved to date do not show clear differences between the species inducing common and dwarf bunt. Band patterns and sequences of amplicons are often not homogenous or reproducible. Further research work has to be done and alternative approaches such as genome sequencing will be performed.

Integrating the effects of sampling and analysis to produce sampling plans for seed-borne pests and pathogens

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In real seed lot testing there may be a number of challenges which affect our ability to detect a specific average level of pest or pathogen with a high probability:

- The prevalence of pest or pathogen may vary between different locations in the lot
- There may be clustering of the pest or pathogen at small scales (between-seed variation)
- The analytical test for the presence of the pest or pathogen will not be perfect

In order to deal with these challenges we can:

- Change the number of primary samples
- Change the size of the working sample
- Change the number of sub-samples in which the working sample is analysed and the analytical method used

Statistical models were produced and used to estimate simultaneous effect of all of these factors on the power of sampling plans to detect the presence of pests and pathogens. The presence of four seed-transmitted pathogens (*Ditylenchus* spp. - *Ditylencus gigas* and *Ditylenchus dipsaci* in *Vicia faba*, *Xanthomonas campestris* pv. *campestris* in *Brassica* spp., *Fusarium* spp. - *Fusarium graminearum* and *F. poae* and *Tilletia caries* in *Triticum* spp.) was studied in a number infected seed lots in a European project: TESTA. The study determined both between-location and within-location variation in the prevalence of the pest/pathogens for each seed lot. We used the models to estimate the minimum number of primary samples to be taken from the seed lot, the working sample size and the number of sub-samples to be tested to meet specified targets for limit of detection of pests and pathogens. Practical sampling plans are described for each pathogen using different targets for limits of detection. The influence of the diagnostic method(s) used and its limit of detection on the size of the working sample required is an essential element of the sampling plan and are discussed.

Umbel browning and stem necrosis in carrot seed production in France: isolation and characterization of the fungal pathogen

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Since 2007, symptoms of umbel browning and stem necrosis have been regularly observed in carrot seed production areas in France. Triangular necrotic lesions appeared on carrot umbels that later spread to the entire umbel and often progressed on to the stems. Diseased umbels became dried prematurely, compromising seed development. The loss in seed production was estimated at approximately 8% of the harvested carrot umbels. The disease resembles the lesions on carrot umbels caused by *Phomopsis dauci* and described in the Netherlands in 1951 (1), suggesting the re-emergence of the pathogen. In our study, more than one hundred strains were isolated from lesions developed on carrot or parsley from fields of seed production located in different French geographical areas. These isolates were characterized according to morphological criteria (mycelial growth, production of pycnidia and size of conidia). Pycnidia produced alpha- and beta conidia that were typical of the genus *Diaporthe* (2). In order to confirm the identification at the genus level and determine the species, molecular criteria (sequences in the ITS regions of the ribosomal DNA) were used. Results showed that 75 % of isolates belong to the *Diaporthe angelicae* species. To determine if this fungus is seed-transmitted, experiments have been carried out on seed lots. For each lot, unprocessed seeds and cleaned seeds were analyzed. Moreover, the plant-to-seed transmission and the seed-to-seedling transmission have been studied to determine if *D. angelicae* could be transmitted to and by seeds.

ORAL SESSION 3 – ENVIRONMENTAL EFFECTS ON SEED QUALITY

Keynote Presentation: Effects of the maternal environment on seed quality

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Seeds are pivotal to agricultural productivity and ecosystem conservation, yet there remain substantial gaps in our understanding of the critical role that the environment plays during seed development and storage, and its effect on seed quality. The looming challenges of climate change and food security require new knowledge of how stress impacts on seed quality, as well as a re-appraisal of optimal storage conditions. These issues are addressed in an EU-funded project entitled "Impacts of Environmental Conditions on Seed Quality" (EcoSeed). Here we report on the effects of the maternal environment on seed quality in four plant species, *Arabidopsis thaliana*, *Brassica oleracea*, *Hordeum vulgare* and *Helianthus annuus*. Seed quality is defined as the sum total of all seed traits that are acquired from seed development on the mother plant to seed germination, including seed vigour and viability, maturity and desiccation tolerance, longevity and dormancy, and the commercial estimates seed yield and 1000-seed weight. Plants were subjected to suboptimal temperatures or drought. Changes in water availability and temperature were close to those predicted in climate change scenarios but were not detrimental to the plants or to seed production. Our results so far indicate that the maternal environment affected plant and seed physiology in a species- and genotype-specific manner. However, both stresses applied individually impacted strongly on seed yield. Further effects of drought and temperature stress were not entirely consistent across the four species tested, whereby *H. annuus* and *H. vulgare* were less affected by the applied stresses than *A. thaliana* and *B. oleracea*. Temperature stress had the strongest effect on the species tested: elevated temperature during seed production led to reduced thermodormancy in *A. thaliana* and *H. vulgare*, and affected mean seed size and quality in *A. thaliana* and *B. oleracea*. Currently,

a set of biochemical and molecular approaches are being applied with a view to defining regulatory switchboards that underpin the seed phenotype, and selected results are presented.

The effect of environmental conditions during seed production on seed development and vigour

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Environmental conditions during seed production significantly affect seed quality – in particular seed vigour. Seed development and germination were analysed in perennial ryegrass (*Lolium perenne* L.) cv. Calibra and tall fescue (*Festuca arundinacea* Schreb.) cv. Tomahawk grown under restricted soil moisture regimes in a four-year pot experiment. In addition germination was examined in a range of commercial perennial ryegrass seed samples. Seed development was characterized by measuring caryopsis length and germination was characterized by measuring early radicle development in seeds of contrasting caryopsis length. In addition radicle length was analysed during the early stages of germination in the commercial seed samples and compared to the number of normal seedlings. Caryopsis and radicle length were analysed using the multispectral imaging device VideometerLab instrument (Videometer A/S) and the number of normal seedlings was determined during a standard germination test. Among the grass species soil moisture had a significant effect on seed development in perennial ryegrass whereas tall fescue was hardly influenced. Further our results indicate that early radicle development is correlated the number of normal seedlings. Our findings will be presented and discussed in relation to environmental effects on seed quality and in particular to seed vigour and further imaging will be discussed as a potential new tool for seed quality assessment. The results are generated as part of the collaborative project “SpectraSeed” between DLF, Videometer and Aarhus University.

Effect of simulated rainfall at different stages of wheat seed development and maturation on subsequent seed longevity

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Poor wheat seed quality in temperate regions is often ascribed to wet seed production conditions. We investigated the possible effect of simulated rain (ear wetting) or rain shelter at different stages of seed development and maturation on subsequent seed longevity in wheat (*Triticum aestivum* L.) grown in the field and modified-environment studies over five seasons (2008-2012) in the UK. Serial harvests were taken throughout seed development and maturation. Subsequent seed longevity (p50, 50% survival period in experimental hermetic storage at 40°C with c. 15% moisture content) increased during seed development and maturation, not attaining maxima until well after mass maturity. The extent of immediate damage to subsequent longevity varied in scale depending upon the timing of simulated rainfall: for example, a 1-4 day delay in the pattern of seed quality improvement early in development, but greater damage later in maturation. Delaying harvest after treatments provided evidence of recovery in subsequent longevity – indicating that the potentially deleterious effects of rainfall to wheat seed crops on subsequent seed longevity may be reversible in full or in part, at least in the absence of pre-harvest sprouting. The extent to which such damage could be reversed by delaying harvest was also sensitive to the stage in development and maturation when simulated rain was provided.

Comparison of the effects of high temperatures during grain filling on grain composition and seed quality of wheat, oilseed rape and pea

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In European countries, periods with high maximum temperatures in late spring (>25°C) will increasingly occur during the grain filling period. Smaller grains and changes in grain composition may occur and impact grain uses and also seed germination. We wanted to compare the effects of high temperatures during grain filling on grain species which accumulate different major compounds: starch, oil or proteins. We measured grain composition as well as seed germination and storage ability, which are often suggested to be correlated. Elevated temperatures were applied during grain filling in greenhouse conditions. While C and N contents remained remarkably unchanged, all the seeds underwent large changes in their composition and also in germination characteristics: for instance, more C18:1 and C18:2 in oilseed rape, lower albumin and higher vicillin contents in pea; decrease in starch content in wheat. Changes in seed germination depended on species: accelerated germination in wheat, even leading to pre-harvest sprouting in oilseed rape; but in pea only increased rates of not germinating hard seeds. Both changes in composition and germination are dependent on ABA content which was strongly decreased under high temperatures when measured on oilseed rape. Finally, the changes in grain quality under heat stress were not just negative, with a decrease in quantity but also improvements in composition and compounds' balance. Seed germination and storability were always lessened.

Influence of seed production environment for oilseed rape germination and vigour

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Oilseed rape is the most important domestic source of vegetable oil in Europe. Its production potential is largely based on the use of hybrids. Due to the increase of extreme weather

events a higher expression of abiotic stress as risk factor for seed quality in seed production is experienced by breeders and yet even higher to be expected in future. The on-field determinants for germination and vigour are complex and depend - besides the genetic disposition of parental components - on environmental interactions from flowering to harvest and on drying and storage conditions. The European consortium CONVIGOUR represents a transnational effort to attain a comprehensive systems-level understanding of OSR seed vigour, its response and adaptation to the environment. During the project phase a wide array of cultivars and breeder lines were evaluated for their genetic diversity in germination and vigour. A number of extreme genotypes were selected and tested in field trials at multiple locations in Germany and France for three years. Field emergence was determined by counting the number of plants per plot after two and four weeks. Two distinct seed productions were used for observing variation in physiological seed traits from laboratory to field. In the first testing year breeder lines were selected from various production years and environments. In parallel they were all multiplied at one location under optimum conditions to be tested in the following years. The results show that genetic expression of germination and vigour can be highly influenced by seed production environment.

Environmental and other factors affecting the quality of wheat seed produced in Scotland

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It is well known that the environment affects seed development and quality. At the OSTS for Scotland we have observed trends in poorer and more variable seed testing results in years with adverse environmental conditions during seed development. Analysis of historical seed testing records for wheat has shown that variation in viability can largely be explained by season of production and variety. Seed viability was higher in years with higher than average temperatures and low rainfall was low. In 2008 and 2012 lower germination results correlated with lower temperature and higher rainfall. Weather conditions over the summer (June to August) better explained variation in viability than annual or spring conditions. This suggests conditions during later stages of seed maturation and at harvest are the most critical for seed quality. In Scotland, high rainfall and low temperatures during seed maturation may prevent drying and inhibit maturation events. Seed survival curves were produced to further analyse sources of variation in seed lots produced in a single year. In 24 wheat seed lots with high germination levels stored at 40°C and 60% RH time taken for viability to fall to 50% varied from 13.2 to 38.9d. Seed grown further south had greater initial viability and more uniform viability loss. Factors affecting seed quality are complex, but through analysis of the considerable data held at the OSTS we hope to better understand variation in quality in order to provide the best advice on how wheat growers can respond to a changing climate.

ORAL SESSION 4 – ISSS COLLABORATIVE SESSION – CELLULAR AND MOLECULAR METHODS AND NEW APPROACHES TO SEED QUALITY DETERMINATION

Keynote Presentation: A systems biology approach to understand and predict seed quality

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Seed development in higher plants is divided into embryogenesis and maturation, which includes organ expansion together with storage reserve accumulation. Besides regulatory pathways involved in these processes, additional pathways are activated during and after seed filling that confer to the seeds the remarkable capacity to survive almost complete desiccation. This trait is an important factor in the preservation of seed viability and quality during dry storage and an essential parameter to ensure fast and homogenous seedling establishment to ensure high yield. Over the past years, we have worked on deciphering the regulatory networks underlying seed survival in the dry state. This presentation will focus on the physiological, biochemical and molecular events that occur during late maturation in relation to the acquisition of seed quality. Using a systems biology approach linking phenotype with molecular events, we will demonstrate how to identify key genes that govern the acquisition of longevity. In addition, we will show how environmental fluctuations during seed development influence the molecular events and leave an imprint on the transcriptome of mature seeds. In analogy with the analytical advances in medicine, these 'omics' approaches will allow for the discovery of molecular signatures to classify phenotypic outcomes in response to environmental stress and to determine the underlying genes governing the phenotypic response.

Discovery of seed longevity genes through genome-wide association mapping using high-density SNP array

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The genebank at IRRI conserves over 120,000 accessions of cultivated and wild *Oryza*. Viability monitoring of seeds stored in the active collection is carried out every 5 years. When the viability of a seed lot in the active collection reaches 85%, testing of the seeds in the base collection, if derived from the same seed lot, commences and a new sample is produced for the active collection. With such a large number of accessions, routine viability monitoring involves thousands of germination tests each year, requiring the destructive use of seeds and incurring considerable financial cost. Recent studies on rice seed longevity has suggested that there are several QTL involved. However, such studies generally use the difference in percentage germination of single seed lots given an accelerated ageing treatment as the measure of

longevity. Hence, they do not take into account the initial physiological quality of the seeds independent of the rate of viability loss. Here, we present QTL/candidate genes associated with seed longevity in an indica rice panel through genome-wide association (GWA) mapping using the high-density SNP array generated from the rice 3000 genome project. Seeds were harvested at different times and storage experiments conducted to get estimates of initial viability and rate of viability loss. For each accession, the sample with greatest longevity was used for GWA mapping. Using the data generated, the genebank will establish a marker platform to develop appropriate testing and regeneration intervals for each accession.

A new spectral imaging based technology for phenotyping and classification of seed samples

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A new technology for phenotyping seeds for seed quality assessment based on spectral imaging is presented. The technology uses spectral imaging together with advanced image analysis to enhance the traits of seed and other material that occur in seed samples. Seed objects are analysed individually and first-level colour, shape and texture attributes are extracted, which are afterwards used to derive second-level attributes involving classification or quantitative measurements of seed traits. The technology uses a vibration unit and a belt to present samples to a spectral imaging device VideometerLab that segments the sample into individual objects. The technology uses a database for training and maintaining the phenotyping of different seed species. Developments have been made, so that it is possible to download phenotype models from the database, as well as upload data back to the database regarding seeds that are either wrongly classified or not recognized by the system, to continuously improve the phenotyping system. The phenotyping technology is demonstrated in an application that measures the purity of spinach seed samples, developed together with two major vegetable seed producers in Denmark. The application classifies a set of well-known seed species as well as unknown material into a number of classes and will be used in production to adjust parameters in the cleaning equipment. Our developments will be presented with focus on the spinach system, and in particular the phenotyping system will be discussed in relation to maintaining an updated inspection system.

A new approach to prediction of normal germination and vigour based on rate of radicle emergence

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Counts of normal seedlings in standard germination tests can take up to 14 days. We present a new approach in which germination and vigour are predicted within 2 days. The mean germination time (MGT) of 12 lots of oilseed rape (OSR), determined by counts of radicle emergence (RE) both manually and by automated image analysis, was highly predictive of germination (% normal seedlings). In 12 other species, a single early count of RE has predicted

MGT ($R^2 \geq 0.90$). Similarly in OSR, manual and automated assessments of RE after 2 days predicted both MGT and normal seedlings (%). In addition, MGT and an early RE count have predicted seed vigour (field emergence) of OSR. In nine radish seed lots, both MGT and RE were highly predictive of normal germination (%), and previously in a further nine lots MGT and a single RE count predicted seed vigour seen in field emergence (MGT, $R^2 = 0.86$; RE, $R^2 = 0.94$) and germination after storage (MGT, $R^2 = 0.92$; RE, $R^2 = 0.88$). RE has also predicted field emergence (vigour) in six other species. Single counts of RE were repeatable in several laboratories for both OSR and radish. Differences in rate of RE will be explained in terms of the so-called ageing / repair hypothesis. This work illustrates how basic observations in seed testing can be developed into automatic and quick seed quality tests. Single and multiple counts of RE can be done using either an automated system or a low cost manual method and could be applied in routine testing to predict both germination and vigour.

The involvement of enclosing maternal tissue layers in seed dormancy

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The seed is a composite of genetic tissues with the peripheral layers predominantly of maternal origin including the pericarp derived from the ovary, seed coat from the integument(s) and the perisperm from the nucellus. All tissue types can be involved in seed dormancy maintenance including physical and physiological dormancy. The involvement of the pericarp and seed coat in physical dormancy is well documented, but maternal tissue layers can also play a role in maintaining physiological dormancy. Examples will be presented to demonstrate that simple physical disruption in the integrity of specific enclosing maternal tissue layers can release the seed from physiological dormancy. In bur cucumber (*Sycios*), removal of the perisperm envelope from dormant seeds results in 100% germination. In gamagrass (*Tripsicum*), the germination unit is a caryopsis enclosed in dried floral tissue (cupule). Removal of the caryopsis from the cupule does not change seed germination percentage, but germination in seeds where the caryopsis layers (pericarp/seed coat/endothelium) are punctured increases germination by >50%. In coneflower (*Echinacea*), the enclosing tissue is a remnant of the integumentary tapetum (endothelium). Its removal improves germination from 35-45% depending on the species. It is possible that in these three representative species, the described maternally-derived tissue layers present a physical barrier to germination or can limit solute movement across the enclosing envelope to participate in control of physiological dormancy.

Effects of seed priming with sodium nitroprusside and trehalose on improvement of chilling and drought tolerance of tobacco seeds and seedlings in relation to proline metabolism

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Nitric oxide (NO) and trehalose are widely involved in the response and adaptation of plants to various environmental stresses. The effects of seed priming with the NO donor sodium nitroprusside (SNP) and trehalose on improvement of chilling and drought tolerance in tobacco seeds and seedlings were investigated in this study. Results showed that seed priming with SNP and trehalose could significantly improve the germination potential, germination rate and germination index of tobacco seeds, shorten germination time, increase the root and shoot length, and fresh and dry weight of tobacco seedlings under normal culture, low temperature, drought, and low temperature plus drought stress conditions. Priming with 100 μmol▪L-1 SNP and 10 mmol▪L-1 trehalose achieved the optimal effects in the present experiments. Further studies showed that SNP priming can increase proline content in tobacco seeds and young seedlings under normal and stress conditions. Priming was shown to enhance key enzyme activities and the gene expression of proline biosynthetic pathways such as Δ1 -pyrroline-carboxylate synthetase (P5CS), glutamate dehydrogenase (GDH), and ornithine aminotransferase (OAT), and depress the enzyme activity and gene expression of proline dehydrogenase (PDH) for proline degradation pathway. These results imply that SNP priming could activate glutamate and ornithine pathways for proline biosynthesis, and inhibit the degradation pathway so that proline could be accumulated in tobacco seeds and seedlings to improve their tolerance to low temperature and drought stresses.

Analyses of several seed viability markers in individual recalcitrant seeds of *Eugenia stipitata* McVaugh with totipotent germination

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Eugenia stipitata has “recalcitrant” seeds, which do not survive desiccation. There is no apparent differentiation between the embryonic axis and the cotyledons, and the seeds also have meristematic potential and can germinate after physical damage. This set of characteristics makes this seed a good model for stress recovery studies because one seed can be cut into sections, and these can be used for several quality tests. In this paper, the germination capacity of seed sections was assessed and, independent of the cut direction, all seed sections were able to develop roots and shoots. Seeds were stored at different environmental moisture contents to induce desiccation stress for 15 and 30 days. In each individual seed, the following parameters were measured: seed moisture content, viability using tetrazolium staining and the antioxidant glutathione (-glutamyl-cysteinyl-glycine), a frequently used stress marker and the results were compared with germination tests using bulk seed lots. A clear distinction could be made between viable and non-viable material at a single seed level, and *E. stipitata* seeds are recommended as good models to study tissue and cell desiccation sensitivity.

ORAL SESSION 5 – CONSERVATION AND USE OF GENETIC REOURCES IN CROP, FOREST AND WILD SPECIES

Keynote Presentation: Conservation and use of genetic resources in crop, forest and wild species in Estonia

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Estonia became a full member of the European Cooperative Programme for Plant Genetic Resources in 1998 and signed the International Treaty on Plant Genetic Resources for Food and Agriculture in 2004. With this Estonia took the responsibility to follow the objectives of the Treaty on conservation and sustainable use of plant genetic resources for food and agriculture.

The activities of conservation and use of plant genetic resources are done according to a National Programme for Plant Genetic Resources. The long-term seed preservation of cereals, vegetables, forage grasses and legumes and *in vitro* preservation of agricultural and horticultural crops are done by the Estonian Crop Research Institute (ECRI). Preservation of fruit trees and berry plants is organized by the Estonian University of Life Sciences. The Botanical Garden of Tartu University preserves medical and aromatic plant species in *ex situ* field collections. All these collections include more than 5000 accessions from 134 species. Expeditions to islands and coastal areas are organised every year to supplement the collections of grasses and wild species.

Activities on forest genetic resources are coordinated by the Estonian University of Life Sciences. Estonia joined the European Forest Genetic Resources Programme in 2003. The biggest activities are in scots pine and norway spruce, but recent activities also include preservation of genetic resources of broad leaved tree species.

Big efforts are put into the sustainable use of genetic resources. Plant genetic resources are studied and intensively used in breeding of new varieties. A good example in this area is the Public Private Partnership for Pre-breeding initiative set up by the Nordic Council of Ministers. ECRI participates in pre-breeding of perennial ryegrass, this includes the extensive use of accessions collected from the wild. A Rural Development Programme and relevant legislation supports the cultivation and spread of historical cultivars and less used crops. Winter rye variety Sangaste, potato Jõgeva kollane and white meliolot Kuusiku are good examples of increased cultivation acreages.

Locally adapted germplasm is used in the restoration of exhausted oil shale mines and peatlands. More than 10 000 hectares of former oil shale mines have now been reforested. Besides the widely used scots pine, test areas with 52 other tree species have been established to find suitable genotypes for increasing the genetic diversity of restored areas. Local genotypes of tall fescue have been used for the recultivation of abandoned peatlands.

Seed storage in *ex situ* genebanks – inter- and intra-specific variation and genetic determination

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Plant genetic resources play a major role for global food security. The most significant and widespread means of preserving plant genetic resources is *ex situ* conservation. World-wide 7.4 million accessions are stored in about 1,750 *ex situ* genebanks. One of the ten largest *ex situ* collections of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, conserving 150,000 accessions from 3,200 plant species and 780 genera. Since the majority of genebank holdings globally are stored as seed, seed storability is of exceptional importance for germplasm conservation. At IPK research on seed longevity was initiated for a range of crops and wild relatives stored over decades. Historical germination data accumulated during 35 years of seed monitoring were analysed to predict species specific seed longevities at IPK storage conditions. The study considered 75 species having at least 100 germination data points. In total 157,402 observations comprising 79,075 accessions were analysed. Beside interspecific differences variation was also detected within species and genetic analyses were initiated using long term stored and experimental aged materials. The complex trait seed longevity was studied exploiting classical quantitative trait locus (QTL) analysis and association genetics. Association-based trait mapping, largely and effectively used in human genetics, is an innovative methodology in detecting genes. We present results obtained for wheat, barley, oilseed rape and tobacco.

Practical aspects of testing seed moisture using equilibrium relative humidity (eRH)

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The relationship between seed moisture and the relative humidity of the air surrounding the seeds has long been recognized as critical to preserving the germination of stored seeds. Seed scientists have constructed isotherms showing how the seed moisture equilibrated to different levels depending on the relative humidity of the storage environment. Historically the focus was on the response of seeds to the environment (i.e. seed moisture as a function of relative humidity) and seed moisture content has been measured using the oven methods or electronic meters that measure electrical current changes in the seed depending on seed moisture content. However, in a closed test chamber the seeds will alter the relative humidity of the chamber air. With the advent of electronic hygrometers it was possible to read the relative humidity of the test chamber at equilibrium with the seed moisture and a new seed moisture test was possible. This concept was first brought to ISTA by Patrick Baldet in 2004 at the ISTA congress in Budapest Hungary. In 2010, Colas and Baldet further explained the concept at the Cologne Congress and at an ISTA workshop in Montargis, France in October that year. The test has been a main focus of seed storage studies for several years now as it describes the biology much more precisely than moisture content. It describes the energy status of the water in the seed and not just how much water is present. The test is relatively easy to conduct, relatively rapid, non-destructive, inexpensive and applicable for almost any orthodox seed. Therefore, it is being used more and more in practical application at seed production facilities and gene banks to measure seed moisture status. Although the test has many attractive features there are a number of issues that can arise in its practical application that must be addressed for the test to be accurate, repeatable, and understandable. Among these factors are ambient temperature, ambient relative humidity, seed size, seed coat, rate of drying, and metrology (i.e. how different relative humidity meters function). The results of laboratory experiments and practical observations on these factors are given as well as success in using the test to prepare seeds for long term storage. The test has great potential for seed management and testing when properly understood and applied. The ISTA Seed Moisture Committee is developing an ISTA rule for this test.

Genebank seed accession phenotyping through spectral imaging

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The T.T. Chang Genetic Resources Center has more than 100,000 accessions of *Oryza sativa* L. For each accession, there is a small sample of seeds taken from the most original material which is stored in the genebank seed file. Each time an accession is regenerated to replenish seed stocks, the new harvest is compared against the sample in the seed file to ensure that the phenotype matches. Highly experienced genebank personnel do this comparison. Recently research has shown on a limited data set that general purpose spectral imaging features can be used to discriminate between seeds of different varieties and hence might be used instead of or in addition to genebank personnel to ensure that the seed phenotype remains constant through generations. This work extends the results to a larger data set and to include imaging features that directly relate to specific phenotypic traits of *Oryza sativa* L. We will use imaging with UV, visual, and NIR wavelengths in the range from 365 nm to 970 nm. Feature vectors combining size, shape, spectral, and textural properties are computed and further processed by machine learning algorithms. Feature selection is used to identify the optimal feature subset, and to obtain general information about descriptive power of individual features and certain feature subsets. The technology may be used in a genebank situation to confirm that seed phenotypes through multiple generations match the most original material. It could also be used to for comparing incoming material with existing accessions to help avoid unnecessary duplication.

How to germinate seeds of bitter gourd (*Momordica charantia* L.) after storage at subzero temperatures

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Bitter gourd is a vegetable cultivated in tropical and subtropical Asia for its immature fruits and tender shoot tips. The Royal Botanic Gardens Kew Seed Information Database classifies bitter gourd as orthodox. However, gene banks reported low germination percentages after storage at subzero temperatures whereas storage at 5°C resulted in acceptable germination percentages. Authors therefore questioned the classification because cold sensitivity is not in line with orthodox seed storage behavior. The objective of this study was to develop a successful freezing and thawing protocol for bitter gourd seeds. Experiments with freezing and thawing speeds between -20°C/min and 0.5°C/min followed by germination tests at 20/30°C were not successful when seeds were cooler than 5°C. However, in a tetrazolium viability test, all seeds were found to be viable after thawing. Also, the embryonic tissues were found to be very firm and dry after freezing. Cold extracted seed oil (stearic acid 40%, oleanolic acid 57%) was found to quickly become solid at room temperature and liquid at temperatures above 38°C. Therefore, after thawing, seeds were kept at 38°C and 100% relative humidity for one day before starting a germination test. After this protocol seeds germinated to 90% even when they were stored at -20°C for one week. These results confirm orthodox seed storage behavior and indicate that a heat-treatment is necessary to germinate bitter gourd seeds after storage at subzero temperatures.

Perspectives on the conservation and restoration of native wild legumes: the biology and ecology of *Astragalus*, *Oxytropis* and *Lathyrus* species as models

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Native grasslands are endangered ecosystems throughout the world mainly owing to anthropogenic activities. To mitigate these losses, the Marie Curie Initial Training Network 'Native Seed Science and Technology' (NASSTEC, www.nasstec.eu) was developed to train 11 early stage researchers (ESRs) and empower the native seed industry for grassland restoration and conservation. This presentation describes one ESR project: the characterization of seed and seedling functional traits *of Astragalus alpinus L.* (alpine milk vetch) and *Oxytropis halleri* Bunge ex W.D.J. Koch (purple *oxytropis*), which are rare and endangered mountain legumes typical of the Scottish Alpine-Atlantic grasslands. While rare, the species are considered indicators of biodiversity in low-nutrient alpine systems, but fundamental bio-ecological data are lacking. The aim of the study was to characterize seeds, seedling roots functional traits and the isolation and molecular characterization of their symbiotic rhizobia with a view to enable re-establishment of native populations. However, *A.alpinus* and *O.halleri* are protected in Scotland and only de minimis sampling of seed is possible. So firstly, a Living Collection was built to provide sufficient seed for characterisation. In the interim, research was progressed using the related legume species *Astragalus danicus* Retz. (purple milk vetch) and *Lathyrus linifolius* (Reichard) Bassler. We report on effective germination conditions and different seed-priming and pre-sowing treatments to enhance germination and seedling-root functional traits.

Water content effect on seed longevity

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Long-term seed storage is crucial to develop ex situ plant conservation strategies. Water content and temperature are critical factors contributing to seed longevity during storage. The effect of water content on seed ageing was studied for a storage period of up to 8 years in different wild and cultivated species. Seeds were stored at different environments comprising a factorial combination of temperatures and water contents. Up to eight water contents were studied, obtained by equilibrating seeds at a range of relative humidities at 25°C, from 91% to 0.5% RH. Storage temperatures ranged from 45°C to ca. -170°C. Seed longevity showed high variability among species. Extreme desiccation at 45°C showed damaging effects to seed longevity for some species, while for others no effect was detected. Lipid content could be related to longevity, but only in some storage conditions. The relative longevity of a species at high water contents did not correlate with that observed at low water contents. The relative position of some of the species as long- or short-lived varied depending on the humidity at which the storage behaviour was evaluated. Therefore, predictions of survival under desiccated conditions based on results obtained at high humidity might be problematic for some species.
Mira S, Estrelles E and Gonzalez-Benito ME. 2015. Effect of water content and temperature on seed longevity of seven Brassicaceae species after 5 years of storage. Plant Biology 17
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Poster Sessions

POSTER SESSION 1 – APPLICATIONS OF GERMINATION AND DORMANCY TESTING

Abstract 1: Mean germination time of accelerated aging and cold tests are the more reliable laboratory vigor tests of maize (*Zea mays L.*) in subtropical and tropical areas

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Maize is cultivated in a wide range of environmental conditions. The lack of tillering in maize makes seed emergence percentage more critical to achieve specific plant density and yield. The cold test and radicle emergence (RE) have been proposed by AOSA and ISTA respectively, for prediction of seed emergence performance in hybrid maize. However in many studies these methods did not have appropriate performance in subtropical and tropical areas, because of cultivation of maize in hot summer. For evaluation of the performance of laboratory tests, it is performed seed production field at two years in four regions of Iran by using three different sowing dates. For evaluation of seed physiological performance and vigor, 17 indicators include warm test, cold test, radical emergence (RE), accelerated aging test (AAT) in different time durations, tetrazolium test, electrical conductivity in different time durations and mean germination time (MGT) in standard, cold and aging conditions were used. For evaluation of seed performance in field, three indicators include field emergence percentage, mean emergence time (MET) and dry weight of emerged seedling was used. According to the results, the only laboratory indicator that had significant correlation with all field indicators in both years was MGT of AAT 96 hours duration. After that, MGT of AAT with 72 hours and MGT of cold test had the most significant correlations. RE test had significant correlations in all field emergence indicators in the second year, but there was just for field emergence percentage in the first year. According to this research, it was concluded that MGT of AAT and MGT of cold test are the more reliable laboratory tests for prediction of seed emergence performance for cultivation hybrid maize in subtropical and tropical areas.

Abstract 2: Evaluation of seed dormancy in one hundred medicinal plants species grown in Southern Iran

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Demand for medicinal plants puts their natural habitats in danger due to over harvesting; hence their domestication is needed. However, seed dormancy can be a problem in their cultivation. Therefore, seeds of one hundred species from 36 families were collected from their natural habitats in Southern Iran (Kerman province) in 2013 to assess their germination. Germination tests were performed on four replications of 25 seeds in H_2O and then dormancy breaking treatments (scarification, H_2SO_4, GA_3, KNO_3 and pre-chilling) were applied on the species that had germination below 80% in H_2O. Seeds of 27 species (e.g. *Ocimum basilicum* and *Salvia macrosiphon*) had high germination (over 80%) in H2O. Dormancy breaking treatments were effective on 64 species (e.g. *Satureja hortensis* and *Ziziphora tenuior*) out of 73 species where germination increased from below 10 to over 90%. In 54 species GA_3, KNO_3 and pre-chilling treatments were effective indicating the possibility of physiological dormancy in the seeds of the majority species.

Abstract 3: Pre-treatment of Norway spruce (*Picea abies* L.) and Scots pine (*Pinus sylvestris* L.) seeds in order to improve seed germination at the last rotation in commercial forest-tree nurseries

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Increased seed germination rate can promote an efficient production process in tree nurseries, especially at later rotations. To decrease the number of empty tray cells at the 3rd rotation (in July), various seed treatments were applied. Twenty-four hours prior to sowing pine and spruce seeds were soaked in solutions of humate GreenOK, dissolved in phytohormone cytokinin, micronutrient compound Nano ELEMENT and in water. Pine seeds were sown in trays with one (I) or two (II) seeds per cell. Seed germination was assessed 10 and 20 days after sowing, and seedling height measured at the end of the vegetation period. Ten days after sowing, soaked spruce seeds had significantly better germination with no pronounced difference among treatments, after 20 days there was no effect of treatment. This initial but not lasting treatment effect on spruce seeds indicated that it is not a requisite for spruce seeds. The effect of seed treatment was more pronounced for pine seeds. Soaked pine seeds had significantly better germination than control trays, both 10 and 20 days after sowing; little or no significant differences were observed among the treatments. The number of empty tray cells was reduced by 10% for pine (I) and by 20% for pine (II). Seed treatment improved pine seed germination and increased the quantity of seedlings at the last rotation. The heights of spruce, pine (I) and pine (II) were statistically significantly different between the variants, but the difference was not considered economically significant.

Abstract 4: A study on the conductivity test in hot pepper (*Capsicum annuum* L.) seed and its relationship with physiological seed quality

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The objectives of this study were to evaluate factors (soaking time, temperature, seed moisture content) affecting results of the conductivity test in hot pepper seeds cv. Andalas and its relationship with seed quality; and to use the conductivity test to detect vigor level based on its correlation with other tests. This study consisted of two experiments, arranged in completely randomized designs with two factors; soaking time (2, 4, 6, 8 h) and temperature (20 and 25°C) in Experiment 1; vigor level (high and medium) and moisture content (8%, 10%, 12%) in Experiment 2, using four replications of 25 seeds. Seeds (10% mc in Experiment 1) were soaked in 25 ml deionized water. The seeds were filtered, and planted using the top of paper method for testing percent germination (PG), speed of germination (SG) and dry weight of normal seedlings (WS). Conductivity of the seed soak water was measured. Data were analyzed using analysis of variance, correlation, and regression. Results showed that conductivity values at 25°C were negatively correlated to WS while at 20°C they were not correlated with any parameters. There were highly significant negative correlations between conductivity values after 4 h soaking or at 8% mc with PG, WS, and SG. Based on coefficient of determination (R2), conductivity values after 4 h soaking were able to explain the values of PG, SG, and WS as much as 86.8%, 58.5%, and 82.2%, respectively, while 8% mc as much as 53%, 49.4 %, and 50.3 %, respectively. Thus, the best method for the conductivity test was using 25 seeds at 8 % mc soaked in 25 ml of deionized water for 4 h at 25°C.

Abstract 5: Pre-sowing seed treatment in *Tectona grandis* to enhance seed germination and seedling growth

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Tectona grandis (Teak) is the most commercial timber in India and South-East Asia. Its large scale cultivation is much hampered by poor germination of its seeds. Successful germination of teak seed is an unsolved problem of the Forest Department. In order to improve germination the present study was undertaken to standardize the collection period and investigate the best pre-treatment of seeds to improve the germination and vigour of fresh and stored seeds. After collection and recording of morphological parameters seeds were tested with 13 different pre-treatments. Of the various pre-treatments tested seed soaking in 20% bleaching powder solution for one hour (T11) proved the best treatment to stimulate seed germination. With this treatment 28, 29, 14, 9, 46, 36, 14, 11, 34, 24 13 and 0% germination was achieved in fresh seed and seed stored for 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 and 33 months, respectively. In control (untreated) seed the germination was recorded as 3, 4, 0, 0, 5, 6, 0, 0, 4, 3 and 0% in fresh seed and seed stored for 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 months, respectively.

Similarly the GVI and seedling length was also found to be promising with T11 over control. In the results of statistical analysis the treatment T11 proved to be the best treatment when compared with all other treatments attempted at 5% probability level. Bleaching powder ($CaOCl_2$) is effective as a pre-treatment as it can regulate the osmotic pressure gradient, allowing just the required moisture to activate enzymatic process and cell division.

Abstract 6: Using isolated embryo growth as an indicator of thermal damage during hot water scarification in seeds with combinational dormancy

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For nursery and forestry operations, hot water treatment for physical dormancy is preferable to acid scarification because it avoids safety and disposal issues. In seeds with simple physical dormancy, a germination test can be used to check thermal damage. However, in seeds with combinational dormancy, it would take several months to access thermal damage because of the time required for chilling stratification. Isolated embryos were used to access viability and vigor in eastern redbud (*Cercis canadensis*) and Judas tree (*C. siliquastrum*) with combinational dormancy. The temperature that provided the highest imbibition and initial embryo viability was 80°C for 5 min and 70°C for 15 min in eastern redbud and 70 or 60°C for 10 min for Judas tree. Initial embryo growth was a good predictor of intact seed germination following stratification. Initial embryo germination was >80% at all hot water treatments, but embryo length was 3-times greater for seeds at 70°C. After stratification, final intact seed germination was >75% for seeds at 70°C, but <40% for other hot water treatments. Stratified seeds scarified at 80°C showed only 37% intact seed germination, but embryo germination was 90%. This suggests that thermal damage at 80°C had a greater impact on seed vigor as indicated by initial embryo growth and the ability to increase embryo growth potential during stratification required for germination. This indicates that initial embryo growth following hot water scarification would be a good quality control indicator of final germination in stratified seeds.

Abstract 7: Seed germinability of 10 species of Hyptidinae subtribe (*Lamiaceae*), found in the Brazilian savannah

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The Brazilian savannah has great biodiversity and an abundance of endemic species, however deforestation is leading to loss of habitats and genetic variability. Several species of subtribe Hyptidinae (*Lamiaceae*), found in this biome are poorly known and physiological characterization is necessary to conserve genetic resources and to form seed banks. In this study we measured the moisture content and tested the germination of ten Hyptidinae species: *Eplingiella fruticosa*

(Salzmann.exBenth.), *Gymneia platanifolia* (Mart.exBenth.), *Hyptis lanceolata* (Poir.), H. *ramosa* (Pohl.exBenth.),H. *velutina* (Pohl.exBenth.), *Mesosphaerum irwinii* (Harley), M. pectinatum (L.) *Kunze, M. sidifolium* (L'Hérit), *M. suaveolens* (L.)*Kuntze* and *Martianthus leucocephalus* (Mart. exBenth.). The seeds were collected in Chapada Diamantina and in the Horto Florestal de Feira de Santana – Bahia – Brazil. The moisture content was determined by measuring the difference between fresh and dry weight of 4 x 100 seeds, dried at 105°C/24 h. Germination tests were done with 4 x 25 seeds in a Petri dish, incubated in a BOD germination chamber, 20-30°C, 12 h photoperiod, 35 days. The seed moisture content of the studied species is suitable for storage. Germination tests showed that Hyptidinae species have different rates of germination dependent on dispersion time, indicating that these species may have different strategies to establish seedlings in the environment. This paper aims to link the work developed by the Medicinal and Aromatic Plants Research Group with the Universidade Estadual de Feira de Santana.

Abstract 8: Optimal temperature for husk tomato (*Physalis ixocarpa* Brot. ex Horm.) seed germination with the top of paper test

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The temperature for standard germination of husk tomato (*Physalis ixocarpa* Brot. ex Horm.) recommended by seed certification manuals, gives results that do not correlate well with those observed under field conditions. The aim of this research was to determine the optimum temperature for husk tomato seed germination with the top of paper test. Three experiments using a top of paper germination test were carried out at the Laboratory of Seed Analysis at Chapingo University, Mexico. In the first trial, seeds extracted from 16 genotypes of different ages, were germinated at 20, 25, 30, and 35 °C. The second trial used seeds of 30 genotypes produced during the same year, and germination tests were performed at four temperatures (25, 30, 35 and 40 °C). The third experiment used seeds of five varieties produced in 2014; germination tests were conducted at four different seed analysis laboratories at 25, 30, and 35 °C. All germination tests were carried out under a completely randomized experimental design, with four replicates of 100 seeds, using a germination chamber Seedburo® for 21 days. The percentage of normal and abnormal seedlings and percentage of non-germinated seeds were evaluated in all experiments. Data analysis was performed using analysis of variance, and Tukey's means tests. Seed age negatively affected physiological quality, and the degree of deterioration was related to genotype. Temperatures of 25 and 40 °C showed low germination. Results obtained suggest that the top of paper standard germination tests should be performed at 30 to 35°C.

Abstract 9: Germination parameters and seed dormancy in different populations of *Avena fatua* in Latvia

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Prediction of emergence is useful for weed control. Seeds of *Avena fatua* often have primary dormancy but it is not uniform in different populations. Mature seeds of *A. fatua* were collected in 41 locations in Latvia in late July – early August. Both intact and mechanically scarified seeds were germinated in laboratory conditions within 14 days to assess possible dormancy. Maximum percentage of germination and time to reach 50% germination were measured. Effect of storage at room temperature and cold stratification on seed germination was tested. Seed morphological characteristics were described and average seed length, mass of 100 seeds, as well as the kernel hull percentage were measured. To monitor emergence and development of *A. fatua* in field conditions trials were established using non-dormant seeds. Seeds from different *A. fatua* populations differed phenotypically, probably due to genetic differences. Germination parameters of intact and scarified seeds also differed significantly. Maximum germination of intact seeds varied from 0 to 50%, while that of scarified seeds varied from 14 to 93%. Time to 50% germination varied from 1 to 49 and from 2 to 47 days for intact and scarified seeds, respectively. Time to 50% germination of scarified seeds negatively correlated with maximum germination ($r = -0.506$), but no correlation was found between germination and morphological parameters. A possibility of using seed germination parameters characterizing particular *A. fatua* populations for adjusting seed germination models based on hydrothermal time is discussed.

Abstract 10: Strategies for removal of hardseededness from small legume seed accessions

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One of the difficulties of seed bank management is related to the fact that seed viability tests should reflect the actual state of the lot/accession and that many lots/accessions must be processed. This aspect is particularly important when seeds display dormancy. Many species of the Fabaceae family have a hard and impermeable seed coat that needs to be scarified. However, the variability within a batch and between batches of the same species (e.g. different populations) can be very high, resulting in damage and/or inefficient dormancy removal. The objective of this work was to study the variability in hardseededness among four populations of the following species: *Medicago polymorpha, Trifolium glomeratum and* T. *subterraneum*. These species have very small seeds that hinder scarification procedures. Different treatments, including sanding and temperature changes, were used. Results varied among species and the most efficient treatment was determined for each one, so that germination increased from 0-10% in control seeds to 84-100% in scarified ones. Using the universal testing machine

TA.XTplus Texture Analyzer, seed hardness was estimated relative to the breaking strength under compressive stress. For each species, great variability was observed within and among populations. The contribution in reducing seed hardness by the best scarification treatment also varied among populations. The usefulness of the hardseededness study to determine the best treatment to achieve the highest germination rates is discussed. Acknowledgments: Work funded by the Spanish Government Project RF2012-00014-C02-02.

Abstract 11: Response of intact seeds of wild rice species to after-ripening and dormancy-breaking chemicals

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Breaking seed dormancy to promote rapid and uniform germination is key to effective and efficient utilization and conservation of wild *Oryza* species germplasm. Dehulling of seeds, though effective, is a tedious process and may present risks of damaging seeds leading to poor germination. Thus effective dormancy-breaking treatments that do not require hull removal such as after-ripening and treatment with dormancy-breaking chemicals are needed. In this study, the response of intact seeds of 16 wild *Oryza* species to after-ripening and dormancy-breaking chemicals was examined. Initial germination tests indicated that the level of dormancy varies among wild *Oryza* species with germination ranging from 0% (strongly dormant) to 57% (weakly dormant). Seeds after-ripened for 21 days at 50°C and non-after-ripened seeds were subjected to a total of 22 chemical treatments which involved germination in 0.001 M potassium nitrate (KNO_3), or pre-soaking at room temperature for 12 or 24 hours in 0.1 M or 0.2 M nitric acid (HNO_3), 1 M hydrogen peroxide (H_2O_2), or 1000 ppm gibberellic acid (GA_3) prior to sowing for germination. A combination of pre-soaking for 12 or 24 hours each successively in either 0.1 M HNO_3 and 1 M H_2O_2, 0.2 M HNO_3 and 1 M H_2O_2, 1 M H_2O_2 and 0.1M $HN0_3$, 1 M H_2O_2 and 0.2 M HNO_3, 1 M H_2O_2 and 1000ppm GA_3, or 1 M H_2O_2 and 0.001 M KNO_3 was also included. Based on the germination results, recommendations on the most appropriate after-ripening and chemical treatments to break seed dormancy for each of the wild *Oryza* species will be presented.

Abstract 12: Evaluation of physiological potential and internal morphology of broccoli seeds by image analysis

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Image analysis of seeds is an effective way for seed quality evaluation of various species, since it is a non-destructive method, quick, has simple methodology and is easy to reproduce. The aims of this research were to relate the density values of radiographic images of broccoli seeds with their physiological potential and evaluate the efficiency of a computerized image system for seed vigor evaluation based on seedling development. Ten lots of cultivar 'Ramoso Santana' were used. The seeds were X-rayed using a MX-20 DC-12 digital Faxitron X-ray, and

submitted to radiation for 20 seconds at 20 kV. The X-rays images of seeds were processed using the ImageJ software to generate numerical gray scale values. After X-ray, the seeds previously identified were tested for germination, and evaluated on the fifth day after sowing. Normal and abnormal seedlings and non-germinated seeds were evaluated by a computerized image analysis software (Seed Vigour Imaging System®), providing the individual seedling length. The gray scales are effective in classifying broccoli seeds according to their internal morphology and association with their physiological potential. Acknowledgements: FAPESP.

Abstract 13: Is the tetrazolium salt efficient to characterize the physiological maturity of maize seed?

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This research was conducted using the tetrazolium salt to identify the physiological maturity of maize seed. The seeds used are hybrid Pioneer 4285, sown in October 2014 and harvest after 40 days after flowering (DAF), with 4-day intervals until 68 DAF. The harvested seeds were evaluated for viability and vigor (germination test, seedling emergence, electrical conductivity test, accelerated aging test and seedling length). The parameters used to determine the physiological point of maturity of the seeds were black layer, the milk line, the dry matter and water content. The evaluation of tissues using the tetrazolium salt was according to the method to assess seed viability, but were also evaluated the activity of cells of the chalaza tissue and endosperm tissue to embryo transfer. Germination was close to 100% and there was no difference between the harvest seasons. The maximum vigor was detected eight days before the maturity point (PM). The physiological maturation was identified at 56 DAF and associated stages of development of the black layer 5 and the milk line 4, the maximum accumulation of dry matter when the seeds had mass of 308 mg and 32% water. The activity of endosperm cells is related to other PM indicators. The tetrazolium salt is efficient to characterize the physiological maturity of maize seed.

Abstract 14: A survey of the scientific articles published in the Journal of Seed Science in the period 1994 to 2014

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Specialized scientific journals are the means used for recording research results, and contribute to the evolution of science. This survey aimed to analyse the technical and scientific production of the articles and scientific notes published in the Journal of Seed Science (formerly known as "Revista Brasileira de Sementes" – Brazilian Seed Journal), published by the Brazilian Association of Seed Technology (ABRATES) in the period of 1994 to 2014. The study involved the distribution of the most published subjects and species, the number of articles by research institutions, national and international universities. 57 issues of the journal were surveyed, encompassing 1413 articles. The main subjects studied were: seed germination and viability; seed vigour; seed production, harvesting and seedling emergence; seed storage and

preservation; seed pathology. Among the most reported species were forest species, soybeans, corn, common beans and fruit trees. The main institutions that contributed to the development of the research were: a) national universities: Federal University of Pelotas (UFPel); Federal University of Lavras (UFLA); Federal University of Viçosa (UFV); University of Sao Paulo (USP/ESALQ); State University of Sao Paulo (UNESP-Jaboticabal); b) Embrapa units: Embrapa Soybean; Embrapa Maize and Sorghum; Embrapa Semi-Arid; Embrapa Vegetable Crops; Embrapa Genetic Resources and Biotechnology; and c) state research institutes. The majority of scientific production is concentrated in the universities of Southeast and Southern Brazil.

Abstract 15: Effect of potassium nitrate, sulfuric acid and stratification on germination of hawthorn (*Crataegus pseudohetrophylla*) seeds

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The genus *Crataegus* L. belongs to the Rosaceae family which has medicinal and ornamental applications. Hawthorn seed germination is poor due to a stony endocarp and embryo dormancy. This study was carried out to determine methods to overcome seed dormancy in *Crataegus pseudohetrophylla* seeds. For this purpose, seeds were sown in plastic pots as a factorial experiment in a randomized complete block design (RCBD) with 3 replications. Treatments included chemical scarification in KNO_3 at 0, 2500, 5000, 7500 and 10000 mg/L and concentrated sulfuric acid (H_2SO_4 96 %) for 0, 30, 45, 60, 90, 120, 150, 180 and 210 minutes followed by either a continuous regime (1 month at 23°C then 3 months at 4°C) or an alternate regime (1 month at 4°C then 2 weeks at 23°C then 1 month at 4°C then 2 weeks at 23°C then 1 month at 4°C) were applied. Results showed that in KNO_3, the highest percentage germination (19.33) and speed of germination (4.18) was determined at 10000 mg/L + alternate stratification and in sulphuric acid, the highest percentage germination (55) and speed of germination (8.63) was determined for 120 minutes + alternate stratification. The highest mean time of germination in both of scarification treatments was observed with the continuous stratification regime.

Abstract 16: The effect of fruit maturity and seed moisture content on seed germination in *Carica papaya* L.

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The current study aimed to evaluate the effect of fruit maturity and seed moisture content on seed germination in *Carica papaya* L. via the germination test of which a validation report is in progress. Flowers were pollinated continually for 9 weeks from 8 December 2013 and fruits harvested 22-30 WAP (weeks after pollination) on 10 July 2014. The moisture content of fresh seeds ranged from 57.4% - 66.2%. At 30 WAP fruits were mature, with yellow fruit and tangerine coloured flesh, at 22 WAP seeds had turned black. The germination of seeds harvested at 30 WAP and sown when fresh or after drying to 10% or 8.8% moisture content was above 93.5% and not significantly different. Germination was severely decreased if fruit was harvested before 28 WAP. The germination of fresh seeds from 27 WAP and 22 WAP fruits was 47% and 9.5% respectively. Reducing seed moisture content to 10% could significantly increase seed germination to 75.2% and 66.2 % for 27 WAP and 22 WAP seeds respectively. The germination of seeds dried to 10% was greater than that of seeds dried to 8.8%, which was greater than that of fresh seeds. The desiccation tolerance germination curve was built via a series of seeds with moisture contents of 64.1%, 28.3%, 23.7%, 15.9%, 11.8%, and 6.5% dried from seeds of matured fruits. Germination was not significantly differently among treatments except fresh seeds, and an accelerated aging test revealed the germination of seeds dried to 6.5% was significantly lower among treatments and the highest germination was seen in seeds dried to a moisture content of 10%-30%.

Abstract 17: Improving the seed vigor of bitter gourd (*Momordica charantia L.*) by ultrasonic and dehydration treatment

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Bitter gourd is an important warm-season crop in Asia. In sub-tropical region, spring season cropping is practiced in order to avoid the disease and pests caused by the high temperature and humidity during the summer season and to obtain better profits. The main obstacle of spring crop bitter gourd is a poor germination rate due to seed dormancy as a result of a hard seed coat and sub-optimal germination temperature (20-25 °C). In this study, ultrasonic treatment is tested to replace mechanical scarification for breaking physical dormancy and seed vigor after dehydration and storage is investigated in order to facilitate transportation and storage and improve the seed value. The result showed that ultrasonic treated 'Moon Shine' bitter gourd seed had significantly higher germination than the control. The best germination was 92%, achieved after 20 minutes ultrasonic treatment at 55 °C. In general a higher temperature (55-60 °C) and shorter duration (10-20 minutes) treatment gave a better effect. We also observed that ultrasonic treated seeds have a looser cell structure and larger intercellular space in seed coat sections compared with untreated seed. The dehydration treatment reduced the seed moisture content to that similar to the original seed, and fit for short-term storage. Our results show that ultrasonic treatment can improve the germination of 'Pai Pi' bitter gourd seeds in sub-optimal temperature and that the treatment effect can be maintained after dehydration and 6 months storage.

Abstract 18: Technical training in tetrazolium and pathology testing of soybean seed in Brazil

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A Quality Control System (QCS) is of fundamental importance for ensuring the production of the highest quality seed. The Seed Analysis Laboratory is the centralizing unit of the QCS. Seed analysts have great responsibility for ensuring the performance of various tests and in the decision making process on the destination of the seed lots. A QCS is used as a tool to diagnose trouble spots in the production system and to point out possible solutions to them. In this sense, Embrapa Soybean developed in 1980 the COMDIA: Complete Diagnosis of Soybean Seed Quality, which encompasses the joint application of the germination, seedling emergence in sand, tetrazolium and seed pathology tests at various stages of the production system. Since 1981, Embrapa Soybean has been offering training in the tetrazolium test and, beginning in 1984, the traditional COMDIA Courses: Tetrazolium and Seed Pathology, which in 2015 completed the 64th edition. Through these courses, 1,321 seed testing professionals have received training. In addition, since 2006, Embrapa Soybean has offered 10 editions of the Vigour Course in Soybean Seeds, training 335 seed specialists. Additionally, nine training sessions have been offered on the tetrazolium test, comprising 193 participants. In total, Embrapa Soybean has delivered 84 formal courses, training 1,813 technicians, representing an important contribution to seed QCS in Brazil and in several countries. Some of these trainings were offered in other countries such as the United States, Germany, South Africa, Costa Rica and Uruguay.

Abstract 19: Physiological quality, anatomy and histochemical changes during development of carrot seeds (*Daucus carota* L.)

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The determination of physiological maturity and the ideal harvest time are essential for obtaining seeds of high physiological quality. The objective of this study was to evaluate physiological, anatomical and histochemical changes in carrot seeds harvested at different stages of development and establish the most appropriate time for harvesting. Seeds from 'Brasilia' produced in Brasilia, DF, ans were harvested at 14, 21, 28, 35, 42, 49, 56 and 63 days after anthesis (DAA). The anatomical and histochemical characterization of the seeds were processed according to the usual techniques in light microscopy and the sections were stained with Toluidine blue, Xylidine Ponceau (XP), Lugol, Sudan Black B. Seed moisture content, dry weight, germination, first count, accelerated aging, and speed emergence index were evaluated. At 14 DAA, the division and cell expansion phase was observed. In addition, substances such as lipids were identified in the endosperm, despite the low dry matter content. At 21 DAA, protein and starch were also identified. At this stage the embryo occupies a small cylindrical region and the integument is a single layer of cells. Maximum dry matter content occurs at 35 DAA. At this time, the seeds have 56% moisture content and the color of the pericarp is yellow-green. Maximum seed germination and vigor occurred at 30 DAA, just before physiological maturity is reached. Thus, the ideal time to harvest the seeds is from 35 DAA, once they have reached maximum dry matter content and physiological quality.

Abstract 20: Use of multispectral images and chemometrics in tomato seed studies

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During the production of tomato seeds, green tomatoes are normally discarded before seed extraction irrespective of their maturity stage. Studies indicate that seeds from green tomatoes may reach be able to reach full germination capacity. Thus the potential of multispectral imaging for non-destructive discrimination of seeds based on their germination capacity was investigated. A total of 840 seeds extracted from green and red tomatoes were divided into two sets; a training set and a test set consisting of 648 and 192 seeds respectively. Each set consisted of 96 seeds from green tomatoes. The multispectral images of the seeds were captured and normalized canonical discriminant analysis was used to analyse the images. Germination tests were performed and seeds that subsequently germinated were recorded as viable. The viable seeds were classified with 99% and 98% accuracy for the training and test set, respectively. Similarly, dead seeds were predicted with 98% of accuracy. Results also showed that 23 and 14 seeds from green tomatoes in the training and test sets respectively were viable, while only one viable seed in the test set was misclassified. The results indicate that green tomatoes might be mature enough to contain viable seeds. However, it still needs to be investigated how these seeds perform in normal growing conditions.

Abstract 21: Dormancy breaking as a prerequisite for using field pennycress (*Thlaspi arvense* L.) as renewable source for plant oil

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Field pennycress (*Thlaspi arvense* L.) belongs to the Brassicaceae family and is a well-known weed in Europe and temperate North America. Since the plants have a short vegetation phase of 2.5 - 3 months only and the seed oil content is about 29%, field pennycress became attractive as an oil crop in double-cropping systems. The oil is rich in erucic and linoleic acids and is usable only for industrial purposes like biodiesel. Seeds of field pennycress show various levels of dormancy causing problems in crop establishment and with volunteers from shattered seeds. Dormancy mechanisms are similar to those found in *Arabidopsis* and *Lepidium*. In this project we evaluate the potential of selected methods to break dormancy. Twelve seed populations

were collected in southwest Germany in the year 2014 in spring and late summer. A tetrazolium viability test method was developed following the principles of the ISTA Rules. Seed viability was usually above 90%. Without applying any method for breaking dormancy, germination at 20°C was between 5% and 25%. Acid scarification (concentrated sulfuric acid for 20 sec) improved germination percentages in most samples. Removing seed coat did not achieve germination of all viable seeds, but removing seed coat and the thin layer of endosperm did. Further tests were made using mechanical and enzymatic scarification, pretreatments with KNO_3, GA_3, GA_{4+7}, Ethylene, Ethephon, prechilling, preheating, and germination in plastic bags. The methodology of viability testing and the most effective methods for dormancy breaking will be described.

Abstract 22: Origin of variation in spinach germination results between laboratories

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Results from spinach (*Spinacia oleracea* L.) germination tests are often significantly different when performed by several laboratories. The high variability of these results can create conflicts between seed companies, producers and reference institutes. Often, a poor correlation exists between laboratory results and field performances. In spinach, an undeveloped rooting system is believed to cause poor field performances. Thus, 8 seed companies started a project in 2011, in cooperation with the Naktuinbouw, to identify the origin of the variation in spinach germination results, with attention to the rooting system. The root characteristics and plants phenotypes were correlated and standard root criteria in laboratory germination tests were used to decrease the results variability. We found a strong positive correlation between root length (RL) and Fresh Weight (FW) and the majority of plants with a growth delay had a 'short' root (Root:Hypocotyl <1). Three ring tests were organized: the first one (RT1) with free germination method and assessment criteria, RT2 with standardized germination method (PP10, 14DAS) but free criteria and RT3 with both standardized method and criteria. There was a high variability in germination results for RT1 and RT2, which was significantly decreased in RT3. We proved that standardized root criteria during spinach germination tests could increase results uniformity between laboratories. Creating a separate 'short root' quality category would improve the reliability of laboratory tests to predict field emergence and meet seed companies' needs.

Abstract 23: Establishment of a Tetrazolium testing procedure for papaya (*Carica papaya* L.) seed

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Papaya (*Carica papaya* L.) is an important fruit crop and the main kind of fruit seed exported from Taiwan. A germination testing proposal for papaya seed had been submitted to the relevant technical committee of the International Seed Testing Association (ISTA) but the tetrazolium testing of papaya seed is not included in the International Seed Testing Rules. This study tested the tetrazolium testing conditions of papaya seed to quickly estimate seed viability. We tested different papaya seed tetrazolium testing procedures including different premoistening times (18, 24 and 48 hours), staining solution concentrations (0.5, 1.0%) and staining times (3, 4, 5, 6 hours). The results show that taking the embryo with forceps and dissecting needles after 18 hours premoistening, and then staining in 1% tetrazolium solution for 3 hours is the optimum procedure for papaya seeds. We evaluated 7 papaya seed lots by studying staining patterns and tissue soundness and separated seeds into to 6 staining types to compare them with germination. The results showed that a combination of type 1 (radical and cotyledons well stained), type 2 (radicle at least 2/3 stained and cotyledons well stained) and type 3 (radicle well stained and cotyledons at least 1/2 stained) calculated the lowest Root Mean Square of 3.83 indicating that these types of staining patters indicate seeds with good germination.

Abstract 24: Computerized image analysis of seedling for vigour evaluation of onion seeds

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Several computational techniques have been proposed to evaluate the seed vigour and among them is included the computerized image analysis of seedlings. To detect vigour differences among lots of onion seeds the efficiency of a computerized imaging system was evaluated. Five seed lots of hybrid 'Bella Vista' were used. Eight samples of 25 seeds for each lot were sown on blue-blotter paper moistened with 2.5 times the mass of the substrate and placed at 25 °C. At 5 and 6 days after sowing (DAS), seedlings were scanned and analysed using the SVIS® software (Seed Vigour Imaging System) and the seedling length, growth, uniformity and vigour indexes were obtained. Other tests traditionally used to evaluate the vigour of onion seeds carried out were germination first count, accelerated aging and electrical conductivity. Seed lots showed similar performance in both seedling age evaluations (5 and 6 DAS). It is concluded that the seedling length and the SVIS® indexes are able to classify seed lots similarly to the traditional vigour tests.

Abstract 25: Fruit classification of Swingle citrumelo using RGB colour parameters in association with seed physiological potential and storability

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The establishment of an optimal fruit ripening stage to seed extraction is very important for the nurserymen to produce seeds of high germination and vigour. The main objective of this research was to evaluate the efficiency of the red-green-blue (RGB) colour measurement system to identify Swingle citrumelo fruits with maximum seed physiological potential and

storability. Fruits harvested at three ripening stages identified by green (G), greenish-yellow (GY) and yellow (Y) pericarp colouration were photographed and the images processed using the ImageJ software. Data were expressed as the average intensities per pixel of R, G and B colour components and a medium RGB pixel value (R+G+B/3). Seeds were submitted to physiological potential evaluations (germination and vigour) at 0 and 5-months storage (packed up in a kraft® paper bag wrapped in a water resistant polyethylene bag of 0.01 mm in thickness and kept in a cold chamber at 5 °C and 65% RH). Quantitative changes in the intensity of R, G and B colour components were efficient markers of the fruit ripening stages. The R component provided the best results to identify each G, GY and Y fruits. Nevertheless, the G component and mean RGB pixel value allowed the classification of G fruits. Fruit ripening stages showed significant effects on physiological potential and seed storability. Seeds extracted from GY fruits showed higher storage potential compared to G and Y fruits. Precise fruit classification may be obtained using the RGB colour system to extract seeds with high physiological and storage potentials.

POSTER SESSION 2 – ADVANCES IN SEED HEALTH TESTING FOR ENSURING QUALITY DURING SEED PRODUCTION AND STORAGE

Abstract 26: The effect of treatment with fungicide and pesticide on the germination and vigor of stored hybrid corn (*Zea mays L.*) single cross hybrid KSC704 seed

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This work was conducted to study the effect of fungicide and pesticide treatment on stored hybrid corn single cross KSC704. The experiment was set up in the seed analysis laboratory of SPCRI at Karaj as a factorial experiment based on a completely randomized design with four replications. Flat, medium and round seed size and shapes were treated with two fungicides Raxil (Teboconazol) and Carboxin Thiram and Imidachlopride (Gaucho) insecticide, as sole treatment combination of fungicides and insecticide including Raxil + Gaucho, Carboxin Thiram + Gaucho and not treated seeds as control. Then seeds were stored at room temperature (25°C) and 5°C in the SPCRI seed sampling unit storage and cold storage respectively. Seeds were sampled three times at 2.5 month intervals. The standard germination test, based on the ISTA protocol, and cold and accelerated aging tests were carried out and percentage normal seedlings, mean germination time, germination rate and seedling length and weight vigor indices were measured. Results showed that percentage normal seedlings and mean germination time increased, but seedling length and weight vigor indices and germination rate slightly decreased in all treatments. Following accelerated aging percentage normal seedlings and seedling length and weight vigor indices increased in all seed treatments in comparison to the control, particularly in seeds treated with Raxil and Raxil+Gaucho. After a cold test, all determined traits decreased in treated seeds, but the decrease was lower in seeds treated with Carboxin Thiram and Carboxin Thiram + Gaucho. Seeds of all sizes and shapes treated with Carboxin Thiram and Carboxin Thiram + Gacho stored at 5°C showed increased seed germination and vigour compared to other studied treatments.

Abstract 27: Evaluation of osmotic potential, electrical conductivity and pH measurements as indicators of store pest damage for wheat and barley seeds

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Damage of two store pests *Rhyzopertha dominica* (Fabricius) (Col.: Bostrichidae) and *Sitophilus oryzae* L. (Col.: Curculionidae) was evaluated on seeds of nine wheat: Shiraz, Aflak, Bam, Homa, Sardari, Falat, Sepahan, DM and DW and two barley: Nosrat and Kavir cultivars. Seeds were classified as sound or damaged. Both damaged and sound seeds were examined to determine osmotic potential (yπ), electrical conductivity (EC) and pH under lab conditions of 28 ± 1 °C, photoperiod 16:8 h, 45 ± 5 RH%. Results showed that *Sitophilus oryzae* was the more detrimental pest and seeds of wheat cultivar Bam were the most vulnerable to damage, with high leakage (Mean damage percent = 10.65%, yπ = -0.260 atm., EC = 0.433 $\mu scmg^{-1}$). Seeds of cultivar Kavir were the most tolerant (Mean damage percent = 0.66%, yπ = -0.135 atm., EC = 1.383 $\mu scmg^{-1}$). Osmotic potential (yπ) and electrical conductivity (EC) were both correlated with pest damage. Seed leaching may be indicative of susceptibility to store pests and it might be useful to consider our findings when developing procedures for storage pest management.

Abstract 28: Development and implementation of seed health testing in Seed Certification Programs for detection of pathogenic seed-borne fungi in Brazil

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Over the course of the last two decades Brazilian agriculture has seen significant increases in grain production, but has also faced some sustainability problems mainly as the result of climate changes. To meet the demand of food production, as required by the increasing world population, while maintaining high quality and sustainability, the use of more effective technologies is fundamental in countries like Brazil. Seed quality control is one of the most important aspects for achieving high yields in crops exploited for food production and seed health is extremely important, as infected or contaminated seeds may introduce and disseminate pathogens causing high impact losses. One difficulty of incorporating seed health controls into certification programs in Brazil has been the lack of efficient and viable methods to detect pathogens like: *Sclerotinia sclerotiorum* in soybean and dry bean, *Fusarium oxysporum* in dry bean and cotton, *Stenocarpella* sp. in maize, *Colletotrichum gossypii* var. *cephalosporioides* in cotton, *Corynespora cassiicola* in soybean and other pathogenic agents of economic importance. As a the result of specific investment by the Brazilian Government and some state foundations on research programs over the last six years, more accurate and rapid methods based on molecular techniques have been developed for the detection of some important seed-borne pathogens. For certification programs it is proposed that protocols will be established for applying molecular methods as a first screening step, which will then be complemented by biological methods to indicate the viability of the pathogens and provide final results of seed health testing. By combining field inspection and laboratory testing the health condition of the seed lots of important crops will be ensured. Research supported by CNPq/Capes/Fapemig/MAPA.

Abstract 29: Determination of the *Microdochium* species causing reduction in seedling emergence in spring oat and barley seed in Scotland

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A range of spring oat and barley seed lots with high *Microdochium* infection levels (*M. nivale* and *M. majus*) and low level *Microdochium* infection controls were assessed for emergence in a laboratory experiment. Dead seeds and both abnormal and normal seedlings were then tested for the presence of these *Microdochium* species using ISTA method 7-022. A correlation between seedling emergence and total % Microdochium species was established for both crops (oat seed, R^2=0.635; barley seed, R^2=0.393). Using *Microdochium* species specific PCR primers, colonies of *Microdochium* arising from infected dead seeds and seedlings were identified. All *Microdochium* colonies isolated from affected oats were identified as *M. nivale*. 26% of the oat seeds and seedlings described as dead and abnormal tested positive for *M. nivale*. Barley abnormal seedlings were infected with only *M. majus*, no dead seeds were found to be infected with *Microdochium* species. On barley normal seedlings, *M. nivale* was found only on 0.7%, compared to 4.5% for *M. majus*.

Abstract 30: Viability of *Phomopsis spp.* in treated and non-treated soybean seeds during storage, in two different environments

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Phomopsis spp. is a major seed-borne pathogen causing the discard of numerous seed lots of good physiological quality in seasons when seed maturation and harvest occur under moist and warm environmental conditions. Such conditions are common in tropical and subtropical regions of Brazil. The objective of this study was to evaluate the effects of seed treatment with fungicides and storage environment on the seed quality and viability of the fungus during storage. Ten seed lots, with high levels of *Phomopsis* were selected. Four samples of 2 kg from each lot were treated (two with fungicides: thiabendazol, fluodioxonil + mefenoxan) at dose of

1.25 mL/kg seed, and two untreated (controls). After treatment, 20 samples were stored in a cold room (10°C/50%RH) and the other 20 samples were stored under warehouse conditions. Evaluations were done at 0, 60 and 120 days. Seed quality was evaluated through: standard germination test (rolled paper towel 25°C/7 days); sand emergence (greenhouse), tetrazolium and blotter tests. It was demonstrated that: i) *Phomopsis* spp. loses viability more rapidly during storage under warehouse conditions than in a cold room, and even untreated seeds showed increased germination throughout the storage period; ii) emergence in sand would be appropriate to evaluate the quality of *Phomopsis* infected seed, even without fungicidal treatment, and iii) fungicides are very effective in controlling seed borne *Phomopsis* resulting in higher germination.

Abstract 31: TESTA project: Validation of a detection protocol of *Ditylenchus dipsaci* and *D. gigas* by sieving method

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The detection and identification of *Ditylenchus dipsaci* and *D. gigas* in seed lots are an obligatory part of the sanitary control and regulation of seeds in Europe (the import, export and sale of seed lots to farmers). After a comparison of the performance of the biological and molecular protocols currently used in Europe, the objective was to validate a method that enables the detection of the two pathogens and propose it as an official ISTA and EPPO protocol. Two protocols, filtration and decantation, were compared to detect nematodes after soaking of seeds (to enable extraction and migration of nematode). Both methods allowed nematodes to be detected in infected seeds (Alfalfa and Faba bean) but the filtration protocol using a sieve to collect nematodes allowed a better estimation of the contamination rate. It enables identification based on morphological characters as well as the quantification of nematodes which is needed to provide contamination rates of the seed. Confirmation is allowed through a PCR method. Three PCR protocols were compared (Kerkoud, Esquibet and Wood) in order to evaluate their performances (trueness, repeatability, reproducibility). Results have shown better performances in trueness and sensitivity for Kerkoud whereas repeatability and specificity were better with Wood primers. This sieving method with confirmation by PCR with Kerkoud and Wood primers is currently being validated in an interlaboratory test with 9 participants coming from France, Germany, United Kingdom, USA, Slovenia, and Czech Republic. Results of the validation are to be presented.

Abstract 32: TESTA project: Validation of detection methods for *Phoma lingam* by blotter and media protocols

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The aim of this TESTA project was to validate the replacement of the toxic herbicide 2,4-D with a deep freezing step in the validated ISTA method 7-004. Both the herbicide 2,4-D and the deep freezing step are known to suppress germination of seeds during the blotter test (Mathur and Kongsdal, 2003) allowing better assessment of the infecting pathogen. After some pretests, the performance of the methods (media and blotter) did not show any significant difference in the capacity to detect *Phoma* lingam on seeds. Comparison between the 2,4-D and deep freezing on blotter showed efficiency of both. The three protocols were included in an interlaboratory validation test. In parallel, different confirmation methods (Pathogenicity test and PCR) were developed. According to Liu et al in 2006, primers LmacR and LmacF and different conditions for the pathogenicity test were tested on a collection of *Phoma* lingam isolates. After improvement to the DNA extraction method, the PCR protocol was tested in one lab only, during the comparative test. The best method was chosen through observation of the expected symptom expression: inoculation at cotyledon stage by injection. All the isolates suspected as being *Phoma* lingam were positive and showed characteristic symptom expression. A comparative test of the three methods (media, blotter 2,4-D and blotter deep freezing) is currently on going, with 10 participants from France, Germany, United Kingdom, USA, Japan, Netherland, Australia and Israel. Results of the comparative test and performance criteria of the methods are to be presented.

Abstract 33: Seed quality for reference materials

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Reference Materials are used for the development and validation of analytical detection methods. They can be used as controls in the application of analytical detection methods, for the calibration of analytical equipment, for use in laboratory proficiency testing and are provided to government agencies for regulatory purposes. During the different production steps, the Seed Quality maintenance and testing are essential parameters to be considered. Bayer Methods Follow Strict Quality Controls to ensure that the different formats of Reference Material (seeds, grains, powder, leaves, DNA) reach the appropriate quality standards. Obtaining a representative test portion by following good sampling practice is critical. Exposure risks are determined by the production responsible who validate the testing strategy to be performed. PCR testing is allowing elimination of non-conforming materials. Automated document management system is also securing the activities.

Abstract 34: Effect of storage fungi on soybean seed deterioration under different storage conditions

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A study to assess the impact of storage fungi on soybean seed deterioration under different storage conditions was set up. A factorial experiment based on a completely randomized design with 4 replications was performed at the Seed and Plant Certification and Registration Institute (SPCRI) in 2012 and 2013. The aim of this study was the evaluation of the effect of different seed moisture content and storage conditions on the infection with storage fungi and soybean seed deterioration. Soybean seeds of Williams cultivar were evaluated for infection of *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* and *Penicillium* fungi. Germination and seedling vigor index after six months storage under both controlled conditions (temperature and relative humidity) and also common storage conditions in Moghan were tested. The results indicated that with an increase in seed moisture seed quality decreased and the infection with fungi increased significantly. A higher infection of fungi and greater decrease in seed quality traits were observed in Moghan storage due to higher temperature and humidity compared to controlled storage. This research indicated that soybean seed deterioration increased severely with an increase in storage fungi growth.

Abstract 35: MILAROM project: Study of downy mildew caused by a *Peronospora* sp. on basil in France

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For greenhouse or open field cultivation, basil (*Ocimum basilicum* L.) production represents more than 250 ha in France and 600 ha in Europe. Since 2001, basil crops have been attacked by a fungal pathogen: *Peronospora* sp. (1) which causes mildew. This pathogen causes disease symptoms on the leaves of fresh plants, which make basil unfit for consumption or processing (deep freezing, dehydration). A research project, called MILAROM, was set up in France. Part of this project is to study identification of the fungus on fresh plants and on seeds. In 2015, more than 30 isolates were collected from fields or greenhouse productions located in different geographical areas in France. In order to confirm the identification at the genus level and determine the species, molecular criteria (sequences in the ITS regions of the ribosomal DNA) were used. Morphological criteria were studied to characterize the different isolates. Moreover, virulence and aggressiveness of 6 of these isolates were also studied. To determine if this fungus is seed-transmitted, experiments are being carried out on 40 seed lots for which contamination is suspected. For each lot, grow out and regular screening of symptoms are being performed. Resistance tests have been set up to evaluate the resistance/tolerance of different basil varieties. Different methods of conservation are being tested to preserve isolates on a medium-term and long-term period. Experiments are underway to determine if *Peronospora* sp. could be transmitted to the seeds.

Abstract 36: Studies on the decrease of spore potential of common bunt (*Tilletia caries*) and dwarf bunt (*T. controversa*) spores of wheat in soil considering different crop rotation systems in organic farming

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Common and dwarf bunt of wheat are important diseases in organic farming. High infection levels lead to an increase of the spore potential in soil. 3-years randomized crop rotation field trials were performed at 3 different sites in Bavaria with 4 replicates on fields naturally infested with either *T. controversa* or *T. caries* spores with crop rotation links commonly used in organic farming. The goal of the project was to determine whether it is possible to decrease the spore potential in soil. Brassica species which release thiocyanate compounds after mulching were cultivated to examine whether they can reduce the spore potential in soil. Additionally, the influence of stable manure on bunt spores was tested. Soil samples were taken yearly from each plot and bunt spore potential was determined by extracting the spores from soil by wet sieving and sedimentation steps, followed by filtration and counting the spores under the microscope according to ISTA (WS No 53). Results show a general decrease of spore potential of about 85 % at the T. caries sites and a 50 % spore reduction at the *T. controversa* site. The plots treated with stable manure exhibit a significant reduction of spore density over all three sites. Significant effects for crop rotation variations 3-years grass-clover and fallow (both without soil tillage) were observed at one *T. caries* site with less decrease of spore potential. No significant effects of crop rotation variations could be seen at the other sites. Plots cultivated with mustard did not exhibit a significantly greater reduction of bunt spore numbers.

Abstract 37: Screening of wheat cross material and breeding lines regarding susceptibility for dwarf bunt on naturally infested and organically farmed fields

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In organic farming, dwarf bunt of wheat (*Tilletia controversa*) is a serious disease that can cause high damage in wheat production. In the case of a heavy infestation, the harvested crop can be used for neither food nor feed. Additionally, the soil is strongly infested with bunt spores for several years. As part of the EU project COBRA, in a two years field trial breeding lines and varieties of winter wheat, as well as a standard set of differential cultivars were tested under practical conditions for their susceptibility to infection with dwarf bunt of wheat. Bunt spore potential in soil at the test site in Bavaria was of natural origin. In both years, the wheat lines and varieties were grown in 2 replications in single rows of 10 meters length each with 25 cm row spacing and 600 kernels per row. Susceptibility was evaluated by counting

the number of infected ears per row and the number of spores per kernel according to ISTA Working Sheet No 53. Results achieved by other research workers regarding resistance as well as susceptibility to dwarf bunt infection could be confirmed. A specific virulence pattern was obtained through the standard set of differential cultivars, making it possible to produce a preliminary characterization of the pathogenic race occurring at the test site. Field trials for resistance against dwarf bunt of wheat are somewhat tricky because of the special weather conditions the pathogen requires. In the case of an adequately high natural bunt spore potential in soil, representative results can be achieved under practical conditions.

Abstract 38: Seed treatment with natural products against seed-borne fungal pathogens: five years of lab tests and field trials

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In organic farming, seed-borne diseases represent a critical problem for successful seed production. The use of healthy seeds is the first option available to prevent diseases and reduce pathogen attacks. During the last five years, we have performed studies and trials to develop organic seed treatments with essential oils such as tea tree, thyme, laurel, peppermint, oregano, clove and rosemary oils against the main fungal pathogens transmitted by vegetables (carrot and rocket), legumes (lentil, pea, chickpea, bean), rice and wheat seeds. Suitable concentrations of oils were defined across in vitro and in vivo assays, evaluating fungicide and phytotoxicity activity. The effectiveness of the oils considered the most interesting was verified with greenhouse and/or field trials using healthy and artificially or naturally infected seeds. Two types of treatment: immersion and spray, plus the use of natural filming by adding chitosan and pinolene to the oils were evaluated.

Laboratory results showed a significant effect of the oils on reducing fungal growth both on artificial medium and in vivo on seeds. Even in field and greenhouse trials, the oils tested achieved positive results in protecting seeds and seedlings from pathogens reducing disease incidence, with results comparable to or better than conventional seed treatment (Thiram or Copper). For wheat and rice seeds, best results were obtained by immersion treatments, but the spray also gave good results if oils were applied together with chitosan for coating the seeds. The immersion treatment was not useful for vegetable and legumes seeds because it damaged the seed teguments and reduced germination; spray application with the addition of pinolene, in order to improve the oil activity, adhesion and persistence on the seed surface showed significant reduction of disease symptoms in greenhouse/field trials.

Therefore we can conclude that the essential oils tested could be considered interesting for developing low environmental impact seed-treatments to control seed-borne diseases. The best application (immersion or spray) should be evaluated for each kind of seed to achieve the best performance. A dressing film could be applied to improve the persistence and effectiveness of the oil application, in particular for spray treatments.

POSTER SESSION 3 – ENVIRONMENTAL EFFECTS ON SEED QUALITY

Abstract 39: Effect of environmental factors caused by different years, regions and sowing dates on seed physiological performance and health of hybrid maize (*Zea mays L.*)

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Maize is cultivated in a wide range of environmental conditions. The lack of tillering in maize makes seed emergence percentage more critical to achieve specific plant density and yield. In order to study the effect of environmental conditions on seed vigor and *Fusarium* infection of hybrid maize, an experiment was conducted in three dates of sowing and two years at four major seed production regions of Iran. For evaluation of seed physiological performance and vigor, stress-base tests in laboratory (cold test, radical emergence and aging test) and field tests were used. According to final seed quality (field emergence indicators) Karaj region and second year of production were the best. In the first year, the first sowing date had the highest level of seed quality. But in the second year, despite the lower mean of air temperature, the third date of sowing had the highest level. This was because of the critical stages of seed development (R2-R3) in first sowing date encountered with hot ambient temperatures. Evaluation of three important seed performance indicators (Field emergence percentage, mean emergence time and dry weight of emerged seedling) showed that minor changes in environmental conditions, could affect the field emergence and growth rate. It is observed that relative humidity variation at maturity stages in different years affects seed infection level to *Fusarium* head blight. According to this research, delaying the sowing date and early harvest in the highest possible seed moisture content is necessary for assurance seed quality and health of hybrid maize seeds.

Abstract 40: Assessment of the effect of drought stress on germination and vigor in soybean, oilseed rape and sunflower; a decade of research experience

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Environmental factors during seed formation on the parent plant have a considerable effect on seed germination and vigor. Among crop plants, oil seed plants are important food staple crops and because of their susceptibility to seed deterioration, study of germination and vigor is important. Even when all required factors for growth, development and seed formation on

the parent plant are provided by growers, parent plants can still be affected by environmental stresses. In many regions of Iran where seed crops are produced limited precipitation and severe evapotranspiration conditions often occur during seed formation. The possible effects of these conditions on seed germination and vigor on major oil seed crops, soybean, oilseed rape and sunflower were investigated from 2005 to 2015. The most common commercial cultivars of soybean, oilseed rape and sunflower seeds were produced in the Seed and Plant Improvement Institute (SPII) fields at Karaj. Drought stress was imposed during flowering and seed maturity by irrigation of parent plants after 50, 100 and 150 mm water evaporation from pan class A, as control, medium and severe drought stress respectively. Seed analysis was then carried out in Seed and Plant Certification and Registration Institute (SPCRI) laboratory, to determine 1000 seed weight, density and seed dimensions. Germination and vigor traits such as final germination and percentage normal seedlings, mean germination time, seedling length and dry weight and related indices were determined by standard germination test and various vigor test methods including the cold test, accelerated ageing test, controlled deterioration test and electroconductivity test. Seed physiological responses to drought stress were also studied by determining levels of indicators such as peroxidase, catalase, superoxide dismutase enzymes and malonyldialdehyde. Additionally, some rehabilitation methods such as various priming and other seed treatments were evaluated.

Abstract 41: Using precision farming to improve barley seed quality

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Spatial variation within fields is well-known with respect to cereal growth and yield, but less is known for seed quality. Similarly, intra-field variation occurs in soil and environmental variables and these are expected to affect the crop. These variables may spatially vary leading to management zones in the field. The objective of this paper was to identify the spatial variation in barley seed quality and to investigate its association with spatial variation in environmental factors and the spatial scale over which this variation occurs. Two uniformly-managed, commercial winter barley crops (cvs Cassia (4ha, 2013) and California 9ha, 2014) in SE England were assessed for germination, vigour, thousand grain weight and seed moisture content. The unbalanced sampling design included five spatial scales (1-81 m) with 138-150 sampling points per field. Canopy variables and soil samples were also assessed at each point. Results for the 4ha field are discussed here. Seed quality variables varied more over long (> 20 m) than over short (< 20 m) distances. Correlations of seed quality with canopy and environmental variables also tended to be stronger over the longer distances. The results suggest that predicting, prior to harvest, spatial heterogeneity in quality characteristics such as seed moisture content could enable a seed producer to harvest and dry seeds separately from different parts of the field. Our thanks to the Higher Committee for Education Development in Iraq for a PhD scholarship to Mr Hama Rash.

Abstract 42: Genetic diversity of perennial pasture grasses and legumes in the response to temperature during germination

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Temperature is one of the major factors controlling seed germination and, in the context of climate change, breeding pasture species varieties adapted to new ranges of temperature could be necessary. *Lolium perenne, Festuca arundinacea, Dactylis glomerata, Medicago sativa* and *Onobrychis viciifolia* are the major perennial species in temperate and Mediterranean regions. The objective of this study was to analyse the genetic variability of five species in their response to temperature in the range 5 to 40°C during germination. Eight populations of *L. perenne*, nine populations of *F. arundinacea*, six populations of *D. glomerata*, seven varieties of *M. sativa*, and two varieties of *O. viciifolia*, were evaluated. Four replicates of 100 seeds per population were tested for germination in the dark under eight constant temperature regimes, between 5 and 40°C with 5°C increments. Maximum germination %, initial time of germination and germination rates were estimated. There were significant differences in the germination of species, populations and varieties ($P<0.01$). No germination at all was observed at 40°C for any of the 23 grass populations. The varieties of sainfoin showed poor germination at 40°C while germination of alfalfa varieties was little affected at 40°C. A differential sensitivity to our extreme treatments of 5 and 35°C was observed. This study suggests that high genetic variability exists within species for response to temperature during germination. This variability could be exploited to breed new grass varieties adapted to the future climate.

Abstract 43: Effect of drying temperature and seed moisture content on lipid peroxidation, catalase and peroxidase enzymes activity in soybean seed

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In order to evaluate the effect of drying temperature and seed moisture content on biochemical components of two soybean cultivars an experiment was laid out as factorial based on a completely randomized design (CRD) with three replications in 2013 in Iran. The factors included were cultivar (Williams and L17), moisture content at harvest time (15, 20 and 25 %) and drying temperature (30 and 45°C). The results of ANOVA showed that the level of malondialdehyde was affected by drying temperature. The malondialdehyde leve in seeds dried at 45°C (58.713 nmol g^{-1} seed) was greater than in seeds dried at 30°C. The highest peroxidase enzyme activity (0.5494 µmol H_2O_2 min^{-1} mg^{-1} protein) was recorded in seeds with 20% moisture content that were dried at 30°C, the lowest level of peroxidase was found in seeds with 25% moisture content that were dried at 45°C. Catalase enzyme activity was greatest (1.6482 µmol H_2O_2 min^{-1} mg^{-1} protein) in seeds with 20% moisture content that were

dried at 30°C, with the lowest catalase enzyme activity found in seeds with 25% moisture content that were dried in 45°C . The results show that with increasing seed moisture consent, the detrimental effects of high drying temperature have been increased.

Abstract 44: Effect of various fertilizers, chemicals and hormones on seed production in seed orchards *Tectona grandis*

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Teak belongs to the family *Verbenaceae*. In India the genus is known only by *Tectona grandis*. The most important teak forests in India are found in Madhya Pradesh, Maharashtra, Tamilnadu, Karnataka and Kerala. Teak usually occurs on soils with a pH range of 6.5 to 7.5; below 6.0 it is practically absent and beyond 8.5 it suffers in growth. The demand for Teak is increasing from the Forest Department and private planters, etc. The Forest Department has established many seed production areas and seed orchards of teak in the State. However, the seed productivity in these areas has been found to be much lower than what had been expected at the time of their formation. The present study was undertaken to increase the productivity of quality seeds in teak seed orchards. Ten treatments plus a control were tested in the form of optimum doses and combinations of chemical fertilizers, organic manure and growth hormones in order to enhance the seed productivity in seed orchards of teak. After application of these inputs, the considerable variation in the productivity of fruit under different treatments was recorded. The data reveals that fruit production increased from 18.72% to 119.76% in treated trees compared with untreated trees. The average fruit production under different treatments (T1 to T9) was recorded between 0.348kg and 0.927kg. However, in untreated trees (Control –T0) the average fruit production was 0.422kg. The highest average fruit production per tree (0.927 kg) was found with treatment 1kg DAP + 20kg FYM + 2000 ppm GA_3/tree (T8).

Abstract 45: Husk tomato (*Physalis ixocarpa* Brot. ex Horm.) seed production

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Husk tomato (*Physalis ixocarpa* Brot. ex Horm.) is the fourth vegetable crop in terms of planting area in Mexico, and its production has been increasing steadily from 270,000 tons in 1990 to 660,000 tons in 2014. Associated with an increase in planting area, demand for husk tomato seed has also increased. Researchers at Chapingo University have developed agronomic production technology to obtain husk tomato seeds with high quality. Transplanting date for seed production has to be defined in order to avoid the presence of rain during fruit ripening, to avoid fruit rotting and premature seed germination. Since husk tomato is a crop pollinated

by bees, certified seed field must be isolated by at least 5,000 m in order to preserve genetic seed quality. Seedling bed is recommended since it represents savings of up to 90 % of seed and it produces more vigorous, healthy, and uniform seedlings. Transplanting individual plants reduced plant competence resulting in good quality seeds. Regarding fertilization, seed growers commonly apply 160-40-40, applying all of the phosphorus and potassium at transplanting time and only 50 % of the nitrogen, with the remaining 50 % applied 40 days later. The highest seed quality is obtained from fruits from the first harvest; with an average diameter greater than 4.5 cm. Seeds have to be extracted 45 to 60 days after fruit harvest in order to achieve more than 90 % of seed germination.

Abstract 46: Variation in temperature limits and rates for *Oenocarpus bataua* Mart. seed germination and seedling growth

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Temperature limits for germination provide accurate quantification of germination efficiency. Such estimates generally relate to the earliest phase of the germination response, that of radicle emergence, and few comparisons have been made with the next stage of growth, that of early seedling emergence. We have assessed the thermal parameters for four stages of seed germination and seedling growth (viz. germination button, primary root and 1st and 2nd cataphyll) in the Amazonian palm tree *Oenocarpus bataua* Mart. Germination was observed at eight constant temperatures with four replicates of 25 seeds for each condition. Constant temperatures of 25 and 30°C resulted in the highest final germination in the shortest time period for all four germination criteria. Alternating temperature did not enhance germination and seedling development. The four germination criteria differed in their temperature tolerance. Whilst the germination button formed over a large range of constant temperatures (10 to 40°C), the efficient production of the 2nd cataphyll (i.e., seedling development) over 60 d was limited to 25 – 30°C. These results indicate that the thermal response for growth can vary with developmental stage and that early seedling growth when seed reserves are being mobilised tends to occur over a more restricted range of temperature than radicle emergence.

Abstract 47: Morphological, physiological and biochemical indicators for tobacco fruit harvest

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In tobacco cultivation, there is little information in the literature about the system of seed production, including on markers of harvest maturity. We aimed to evaluate the possibility of using fruit appearance as an indicator of physiological maturity for the cultivars CSC 444 (Virgínia) and CSC221 (Burley), and physiological and biochemical changes in seeds to establish the ideal moment to harvest. Fruits of cultivars CSC 444 and CSC 221 were harvested at different maturation stages and physiological quality was evaluated by percentage germination, first

count of germination, germination speed index, T50, average accumulated germination and seedling emergence. The activity of catalase (CAT), esterase (EST), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), alcohol dehydrogenase (MDH), endo-β-mananase and heat resistant proteins was measured. It was concluded that the fruit appearance is an indicator of maturity and tobacco seed quality. The partially dark fruits of cultivar CSC 444 and dry fruits of cultivar CSC 221 produce seeds with higher quality. The ADH enzyme is indicative of the ideal stage for harvest of fruit cultivars CSC 444 and CSC 221. Keywords: *Nicotiana tabacum* L., physiological maturity, vigor.

Abstract 48: Physiological performance of soybean seeds subjected to the accelerated aging test and germinated after several waiting periods

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The accelerated aging test is an effective test widely used to evaluate soybean seed quality due to its excellent relationship with seed lot storability potential and emergence potential in the field at sowing time. In the test procedures, it is generally recommended that seeds must be set up for germination within one hour of the end of the aging period. The objective of this research was to evaluate the effect of different periods of delay after applying the aging stress, prior to setting up a germination test to determine the effect on the physiological performance of soybean seed. Soybean seeds of cultivars BRS 397, lots 1 and 2, BRS 284, lots 3 and 4 with different vigour levels determined by the tetrazolium test were used: lot 1 with 92%; lot 2 with 86%; lot 3 with 78% and lot 4 with 64%. The seeds were aged in plastic accelerated ageing boxes at 41 °C for 48 hours in a water-jacketed ageing chamber. The treatments were eight distinct periods of delay in hours as follows: 0, 1, 2, 3, 4, 5, 6 and 8 hours of delay prior to setting up the germination test. The experimental design was completely randomized with four replications. Data analysis and interpretation of results allowed the conclusion that there is no significant effect on the physiological performance of soybean seed with a delay of up to eight hours after accelerated ageing, prior to setting up the germination test for high vigour lots of both cultivars.

Abstract 49: Controlled deterioration test of oilseed rape seeds predicts seedling emergence in a large range of field conditions

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Intensive breeding to eliminate some unfavorable seed compounds for both human food and animal feed and to increase grain quality may have changed seed traits related to germination or seed vigour. Seed vigour is a key trait required for predictable seedling establishment and

which includes rapid germination, rapid initial growth and storability. Improvement and prediction of seedling emergence and early seedling growth are important to the seed industry as this plant establishment stage will be conditioning the crop yield. Oilseed rape (*Brassica napus* L.) is a quite recent oil crop emerging from a spontaneous interspecies cross (*Brassica rapa* x B. *oleracea*) and is one of the largest worldwide oilseed crops. A European scientific and technical consortium involving eight partners has phenotyped 735 oilseed rape cultivars and breeders elite lines to evaluate the genetic diversity for germination traits. Extreme genotypes were selected with contrasted germination speed and have been studied in field trials for two years in six German and three French locations. Two seed production were used as both phenotypic and genetic variation in physiological seed traits can affect the field establishment success. The extreme genotypes were also deeply analyzed in the lab for their vigour using controlled deterioration tests or germination under abiotic stress conditions. Several interesting correlations were obtained between lab and field traits: the controlled deterioration test gave the more predictive results for field emergence measured two weeks after sowing.

Abstract 50: Presowing seed treatment with humic substances for improving germination, emergence and growth of spring barley seeds under abiotic stress conditions

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Humic substances affect plant growth directly as well as indirectly. The indirect effect is primarily based on the influence of substances on soil structure, nutrient content and water retention, while plant metabolism is affected directly. The direct effect is often interpreted as a result of hormone-like activity of humic substances, especially as auxins and similar components which are present in their structure. In this study, seed characteristics of spring barley were tested after treatment with the humic compounds. The seed lots were treated with solutions of Energen Fulhum (a modified aqueous solution of salt materials gained by original destruction of technical lignosulphate) and Lexin (a solution of humic acids and fulvic acids with addition of auxin) preparations and compared with untreated seeds and seeds treated with water – hydration treatment (control and so-called wet control). Thus, the effectiveness of seed treatment on germination, emergence and young growth were tested under different stress conditions (drought, cold and combined cold-dry stress). Only Lexin gave an increase in germination rate and growth of root and aboveground biomass in all types of environment. The application of Energen Fulhum had an ambiguous effect, and improvement of vegetative parameters wasn't statistically significant (even in optimal conditions, a decrease of aboveground dry weight biomass was observed), but the germination was slightly faster. Furthermore, treatment with water had also a small influence on germination rate.

Abstract 51: Impacts of environmental conditions on seed quality of soybean

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This research was conducted to evaluate the effect of environmental conditions in the region of production on seed vigor and storability. Soybean cv. Williams seed lots that were produced at Moghan and Golestan provinces and cv.L17 seed lots that were produced at Moghan and Lorestan provinces were sampled. Seed quality tests were conducted for seed lots after 0, 6 and 12 months storage. The results showed significant differences in seed vigor levels between different seed lots. Seeds produced in Moghan had greater storability than the two other seed lots. Cultivar Williams from Golestan had the highest initial quality, lowest percentage of abnormal seedlings and lowest electrical conductivity. The quality of seeds produced in Golestan rapidly declined during storage, whereas the seed lots from Moghan had the highest quality after one year of storage. The seed lot from Lorestan had low initial quality. After evaluating weather information from the production regions, it is suggested that higher humidity and higher rainfall late in the season in the Golestan region caused higher seed deterioration during storage. The seed lot produced in Lorestan had very low initial seed quality due to high moisture levels in pods as a result of unfavorable environmental conditions at harvest and late season rainfall. This seed lot had high electrical conductivity, which indicates high cell leakage, and seed quality reduced during storage. The investigation of seed storage duration influence on seed quality of soybean that originated from different regions showed that an increase in storage duration caused significant losses in seed quality in all seed lots.

Abstract 52: The effect of mother plant planting date and density on germination and vigor of soybean seed under standard germination and accelerated aging tests in two areas (Moghan and Karaj)

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This experiment was conducted as a split factorial based on a completely randomized block design with three replications at two locations in Iran in 2013. The factors studied included planting date (5th of May, 5th of June and 5th of July), plant density (300, 400 and 500 thousand plants/ha) and soybean variety (Williams and L17). The highest normal seedling percentages in seeds grown in Moghan (92.1%) and Karaj (96.2%) were obtained in seeds from mother plants planted on 5th of June, and and on 5th of July respectively. In addition, it was noticed that cv. L17 in Moghan and cv. Williams in Karaj had the highest percentage of normal seedlings. The results of the accelerated aging test showed that the quality of seed produced in Karaj was better than that of seed produced in the Moghan area. There was no significant difference in seeds from the Moghan and Karaj areas planted with 300 or 400 thousand plants/ha, but there was a significant difference in the normal seedling percentage after accelerated aging test in Karaj (77.6%) and Moghan (58%) areas planted with 500 thousand plants/ha. The highest seedling vigor index (11.75) was obtained with a density of 400 thousand plants/ha, planted on 5th of July in the Karaj area and the lowest seedling vigor index (5.41) was observed with a density of 300 thousand plants/ha planted on the 5th of June in the Karaj area.

Abstract 53: Seed vigour, soluble sugars and storage proteins: reciprocal effect of parental lines of maize hybrid seeds

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Maize (*Zea mays* L.) crop has great possibility of producing seeds with high vigour when compatible parental lines are used, and this relates to desiccation tolerance and seed composition. This research aimed to study hybrid maize seeds from reciprocal crosses, attempting to verify if they produce seeds with different quality and to correlate vigour with seed composition, focusing on storage proteins and soluble sugar amounts. Four maize hybrid seeds lots (HS 15, HS 51, HS 24, and HS 42) were evaluated by germination, first count of germination, cold test, accelerated ageing, electrical conductivity, and seedling emergence in the field tests. Seed composition was assessed in dry seeds. Storage proteins were extracted using sequential buffers, quantified and analysed by electrophoresis. Soluble sugars were determined using High Performance Liquid Chromatography with Pulsed Electrochemical Detection. Mean values were compared with Tukey test at 5% level of probability and a correlation analysis was performed between physiological potential and quantity of soluble sugars. L1 lineage used as female parent produced seeds with lower vigour. The quantification of seed storage proteins and sucrose, raffinose, and stachyose were equal between seed lots, while high fructose and glucose amounts were found in lots with low vigour; correlation analysis confirmed these results. It is possible to infer the existence of reciprocal effects of parental lines of maize hybrid seeds and this influences seed vigour and reducing sugar content.

Abstract 54: Seed accelerated aging of rice for longevity evaluation in the humid tropics

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Rice seed of *Phathum thani* 1 and *Cho Lung* 97 varieties were used for this study. Seeds were aged at almost 100% relative humidity at 40, 42, 44 and 46 °C for 24, 48, 72 and 96 hours. Seeds were also stored in plastic bags at room temperature. Every month rice seeds were monitored by quality testing. The quality of rice seed stored in accelerated aging conditions at 46 °C for 96 hours was similar to the quality of rice seed stored in plastic bags in the environment of southern Thailand for 6-7 months in Cho Lung 97 and 4-7 months in *Phathum thani* 1.

POSTER SESSION 4 – ISSS COLLABORATIVE SESSION – CELLULAR AND MOLECULAR METHODS AND NOVEL TECHNIQUES FOR SEED QUALITY EVALUATION

Abstract 55: How does Microtomography provide access to full seed morphology? Current applications and future prospects

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Microtomography (micro-CT) is a non-destructive technique that uses X-rays to investigate the internal anatomy and morphology of organisms. It generates a series of projections reflecting the level of attenuation of X-rays after crossing an object from different angles during its rotation. Projections are then collected and reconstructed to obtain a 3D stack volume. In plant seeds, seed external appearance does not provide sufficient information about seed physical quality. For example, seed may seem unaffected while it can be damaged, cracked, have an abnormal embryo etc. The use of micro-CT can thus assess numerous seed internal and external structures (e.g. embryo, cotyledons, teguments, cavities) visualized in 3D and with a high accuracy (up to 5 micron resolution). It can also be used for the characterization of varieties for different traits or for coated/treated seeds for the physical quality of the treatment. Quantitative information (e.g. volumes, lengths, shapes.) are extracted using image processing algorithms. These algorithms differ from one seed species to another and according to the problematic. Here, we present different applications of micro-CT combined with image processing applied on seeds and the future prospects of this promising technique on seed science and technology.

Abstract 56: GMO screening in maize seed samples: validation of a model multiplex approach

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The high number of GM maize events marketed worldwide is a reality and there is an increasing diversification of the elements composing gene constructs. This complexity requires the introduction of various screening elements marking the presence of transgenic sequences. To detect the adventitious presence of the GM seeds in conventional seed lots the real time PCR is the elective technique and at first a screening can detect a large set of GMOs in a sample. To develop a cost-and time-effective screening assay the laboratory adopted a multiplex approach for the simultaneous detection of 35S promoter and NOS terminator as screening sequences and a maize endogenous gene as reference for DNA quality. This triplex assay allows the detection of almost all the GM maize events authorised in EU up to now. The laboratory conducted an in-house validation study showing the good performance of the method. The PCR assay shows high sensibility and sensitivity and it is able to detect down to 7 copies of each screening sequence (absolute LOD). Likewise the assay detects down to 7 copies of a target sequence even if the other sequences are present in a high amount of copies (asymmetric LOD). The laboratory demonstrated that this screening assay is robust and useful for the seed testing laboratories asked to provide reliable results in a short time for a large number of samples. As the kind of commercialized GM events are evolving, new assays for the screening step could be developed in the near future. The validation process of the triplex method is a suitable model for multiplex approach.

Abstract 57: Study on GMO detection in maize and soybean seed lots: the Italian case

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Since 2003 the Italian Ministry has organised a monitoring programme in order to check the possible adventitious presence of GMOs in seed lots addressed to the Italian farmers. Several public Institutes are in charge of sampling the seed lots and analyze the samples in the aim to verify the absence of GM seeds in the conventional maize and soybean seed lots intended for the national market. They are Fraud Repression Inspectorate, Customs Agency, Phytosanitary Regional Services and CREA-SCS Laboratory that is ISTA accredited for GMO testing. The work presents an overview of the results of more than a decade of controls: as a total, about 18000 samples of maize and 3000 samples of soybean seed lots have been sampled and analysed. The trend of the positive lots over the period is shown and discussed. Positive results are analysed in relation to the overall results, according to the country of origin of the seed. A direct connection between the positive lots and the regions of the world where GMOs are cultivated could be identified. Data are also explored with reference to the detected GM event/s.

Abstract 58: Introduction of SSR molecular markers in the official procedure for the registration of new soybean varieties in Italy

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The Italian official procedure for the registration of new varieties of *Glycine max* (soybean) has been recently revised. Beside the morpho-physiological characteristics of the plant that are evaluated in the field plots, the protocol includes molecular characteristics assessed by the laboratory. This work was carried out during the revision of that guideline. Its aim was to

select a set of markers suitable for varietal characterization, with high level of polymorphism and high accuracy. These features, together with the suitability for routine analysis, would represent an important achievement for the laboratory asked to test and describe new soybean varieties. SSR (Simple sequence repeat) markers were selected. These are codominant, highly reproducible and polymorphic markers; for these reasons they are widely recognized as an easy and reliable molecular tool for breeding programmes and variety registration. At a preliminary stage, 51 SSR markers available from literature and SoyBase public database were selected. Allelic diversity, PIC (polymorphic index content) and chromosomal location were considered for the selection. The 51 SSR were tested on 12 varieties. This was a mini-core collection, representing the pool of varieties cultivated in Italy, e.g. with different earliness. The set of varieties included accessions with both a common and a different genetic basis in the aim to evaluate the discrimination capability of the selected markers. Among the large set, 20 SSR primer-pairs have shown desired features. They are highly polymorphic and well-distributed (one marker for each genetic linkage group). Moreover, they consist of trinucleotide repetition units and their amplification produces a good electrophoresis profile, facilitating unambiguous interpretation of the results. At international level, in the last years an intense discussion on the use of molecular markers in seed testing and for variety registration has been going on within organizations such as ISTA, OECD and UPOV. In soybean, the results of this work contribute to demonstrate the usefulness and the potential of molecular techniques for varietal characterization for official purposes.

Abstract 59: Applicability of early count of physiological germination as a vigour test to predict field emergence of oilseed rape (*Brassica napus* L.)

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Samples of 19 seed lots from 19 farmers, which were cultivated in different areas of the Khorasan province, Iran were obtained and used in physiological germination tests before and after CD test. Seedling emergence percentages of the seed lots in the fields of all farmers were assessed 30 days after sowing using one square meter quadrat at different parts of each field randomly. The relationship between laboratory results (final germination, normal seedlings and MGT) before CD with field emergence was not significant. But, there was a significant correlation between final germination, normal seedlings and MGT after CD with field emergence ($r = 0.57$, 0.51 and 0.49, respectively). Early counts of physiological germination before CD, made 2 days after the beginning of the germination test, were related to emergence performance in the field ($r = 0.47$, $p<0.05$) as well as early counts of physiological germination 2 ($r= 0.55$, $p< 0.01$) and 3 days ($r=0.48$, $p<0.05$) after CD test. These results revealed that the early count of physiological germination is a convenient way to identify differences in vigour and predict field emergence of oilseed rape. This method, called RE test, is a rapid and simple method in comparison to the CD vigour test.

Abstract 60: Determination of the mother plant for Nutmeg (*Myristica fragans Houtt.*) seed production using Random Amplified Polymorphic DNA

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The main purpose of this research was to determine the mother plant of nutmeg using Random Amplified Polymorphic DNA (RAPD). This research was conducted from August 2014 until August 2015. Seeds were harvested from nutmeg plantations in three locations (Toisapu, Wakal, and Lula) in Ambon, Indonesia. RAPD analysis was conducted at the Plant Biotechnology Laboratory, Bogor Agricultural University. The DNA analysis used 21-week-old seedlings. DNA was isolated from fresh leaf tissue. Amplification products were separated by electrophoresis. The RAPD data was analyzed using the NTSys to evaluate the uniformity of seed among the trees. Based on RAPD data and Dice's similarity, UPGM-Cluster analysis has been done. Analysis of the uniformity was also done by the incorporation of RAPD data and morphological characters. The uniformity analysis using morphological characters was done previously. Results showed that the uniformity level of seeds of nutmeg mother plants at Wakal had the lowest uniformity level (0.61-0.93), due to nutmeg tree number 7 at Wakal which had a uniformity level of only 0.61. The uniformity of nutmeg trees at Toisapu, and Lula were 0.90-0.96, and 0.85-0.93. It was indicated that the seeds within and between three locations were uniform. This fact was also supported by joint analysis of RAPD data and morphological characters. Based on this research it can be concluded that all mother plant scan be used for seed production, except nutmeg tree number 7 at Wakal. This RAPD method can be used for determination and selection of mother plants as a seed source with high uniformity.

Abstract 61: Development of molecular markers for purity testing in Thai jasmine rice

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Purity testing in paddy rice is sometimes difficult when using phenotypic characters alone. Molecular markers can be a more accurate technique for purity testing. Simple sequence repeats (SSRs) markers are widely spread throughout the rice genome and are used for purity testing in rice. DNA banding patterns from an automated DNA sequencer showed more allelic variability than those from a polyacrylamide gel electrophoresis technique. An average allelic pattern detected by using an automated DNA sequencer is 9.38 alleles per locus. The marker RM20B showed the highest allelic variation of 20 alleles, while the marker RM165 and Glu-23 showed the least allelic variation of 3 alleles. Those markers that can differentiate the rice varieties KDML105, Pathumthani 1 and Chainat 1 are RM21, RM20A, RM20B, RM209, RM3, GT11, RM232 and RM235. An average allelic pattern detected by using polyacrylamide gel electrophoresis is 4.53 alleles per locus. The marker RM20A showed the highest allelic variation

of 9 alleles, while the markers RM165, B03 and Glu-23 showed the least allelic variation of 2 alleles. Those markers that can differentiate the rice varieties KDML105, Pathumthani 1 and Chainat 1 are RM21, RM20A, RM20B, RM209, RM3, RM248, GT11, RM204 and RM3627. Those markers used for purity testing can be classified into 3 groups; 1) those used for identifying soft and hard cooked rice, 2) those used for identifying glutinous and non-glutinous types and 3) those used for identifying aromatic and non-aromatic varieties.

Abstract 62: Photonics technology for rice seed characterization

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Good rice seed quality is a major factor for rice production. Seed characteristics can be used to identify rice varieties. Seed size is normally determined one seed at a time by a micrometer, which is a time consuming method. A rapid and reliable protocol for rice seed characterization could be developed. In this study photonics technology (which combines optics, electronics and software) was used to increase the efficiency of rice seed analysis. Generally, only the size of a whole seed is measured, while the size of an embryo has never been measured separately. It is well known that the embryo of a rice seed is an important part for rice seed growth and also contains essential nutrition. The aim of this study was to determine the relative size of embryos and endosperms of brown rice seeds and also to study the genetic variation in embryo sizes of rice seeds using photonics technology. An image of brown rice seed was captured under white light illumination and the areas of embryo and endosperm were analyzed. 100 seeds of each of forty-four rice cultivars were measured and it was found that there was variation in the size of embryos and endosperms of brown rice among the cultivars studied. The sizes of embryos of brown rice varied from 4.4 ± 0.129% to 8.54 ± 0.145% and the sizes of endosperm of brown rice were ranged from 91 ± 0.178% to 95.6 ± 0.129%. Moreover, the variation in shape of embryo and a whole brown rice seed among rice varieties were also investigated. These characteristics could be used to identify rice varieties in the future.

Abstract 63: Introscopic techniques to identify internal structural defects of grain and forestry seeds, caused by various environmental effects

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Seeds are exposed to various environmental and technology-related harms during all stages of the production cycle. In these cases seeds have different defects. The aims of this research were: 1) Visualization of some of the internal structural defects within wheat seeds, such as: fissuring, internal germination, fermentative lysis produced by insect (*Eurygaster integriceps*) and others. 2) Comparative study of *Picea abies* L. normal and empty seeds. The projection microfocus X-Ray (PRDU-02) and Micro-CT (Bruker SkyScan 1172) techniques were applied for visualizing structural defects of wheat seeds. The Micro CT study was performed in RC Geomodel, St. Petersburg. Projection microfocus X-Ray and Gas Discharge Visualization (GDV Camera Pro) techniques were applied for the evaluation of normal and empty *Picea abies* L. seeds. The advantages of Micro-CT technique are: resolution allows finding and visualization of all seed defects in great detail; micro CT analysis provides an opportunity for obtaining multitude projections and sections of seed. Advantages of X-Ray technique are: high efficiency (up to 100 grain seeds at the same time) and comparatively high speed of image capture (up to 2 min including scanning). The results indicate that empty seeds of *Picea abies* L. do not show gas discharge glow and show lower level of X-Ray patterns brightness in contrast to normal seeds. Further, the results show that the projection microfocus X-Ray, Micro-CT and gas discharge visualization techniques can be applied as a reliable tool for introscopic testing of grain and forestry seed quality.

Abstract 64: Identification of morphologically similar seeds using computer vision

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Seed identification is currently done manually by specialized professionals and experts with limited aiding tools and references. The most cumbersome and time-consuming part of the task is identifying minute seeds that have similar morphological or visual features among plant species. Using image analysis or computer vision for identifying seeds has been studied and good recognition rates have been achieved. However, the data sets used in previous studies contain few species, lack of the verification of specimens or little representation of intra-species variations. This study considered a data set containing seed specimens that were verified to represent the species and a typical population variation. Look-alike species that share similar seed morphological appearance or seeds from species in the same genus were selected, which can be particularly difficult for even trained professionals to visually distinguish. Three different types of features from seed images: colour, shape, and texture were extracted, and a multi-kernel support vector machine was used as the classifier. Local features at different scales were extracted on dense grids and then encoded and pooled to form the final representation of the input image. It is demonstrated that, by using seed images analysis or computer vision, morphologically similar seeds can be identified with the accuracy of >97% in the proof of the concept study. The features previously deemed “optimal” and the value of texture discounted are not agreed with this study. The texture proved to be an important discriminator in this study for the species used. The study also explored identification possibility with lower quality images taken with a single focus point.

Abstract 65: Development of a non-destructive method for the measurement of endosperm volume of maize seed using x-ray image analysis

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Seed, its embryo, endosperm volume and seed coat sizes have long been discussed as effective factors for determining seed vigor and seedling quality. We have developed a non-destructive method using image analyses which measures endosperm and seed coat volume (vs embryo size). For this purpose, a cassette was used to fix 100 maize seeds (as a replicate) and keep them in a stable form. Four different varieties of maize seeds were studied in 4 replications. For the image acquisition, seeds were located in a cassette and transferred to a computed tomography (CT) scan system. Images were acquired in 2D format and endosperm plus seed coat was separated from embryo in different color by defining a threshold. The image of each replicate was sliced in 0.63 mm and divided into square tiles. 2D images of each slice as a Bmp format were automatically transferred to 3D image by an algorithm written in MATLAB. The measured endosperm and seed coat volume of each seed lot was compared with lab results obtained manually. There was a positive correlation between the new method and the lab result showing accuracy of the new system. The new system is rapid, precise and costly and can be used for the measurement of endosperm volume as well as embryo size of different seed lots.

Abstract 66: Harnessing genomic tools for seed vigour in rice (*Oryza sativa* L.)

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Seed vigour is the most important attribute of seed quality and an important trait for better crop establishment, wherein limited or excess moisture prevails under un-favourable soil conditions. Many high yielding indica cultivars perform well under transplanted conditions but fail to establish under direct seeded conditions. A set of 46 rice genotypes were screened for seed traits viz., seed vigor, grain yield and correlation of seed vigor with root QTLs. The study revealed that genotypic variability existed and high seedling vigour was expressed in Dinesh, Moroberekan, Nipponbare and VL Dhan 208. These varieties also had multiple root QTLs and multiple seedling vigor QTLs indicating that these genotypes have an inherent capacity for early emergence with high seedling vigour and it may be due to the presence of these putative QTLs associated with different seedling vigour traits. We have also successfully finger printed the released varieties of AP using SSR markers and developed functional markers for grain size and grain number. The genotypes with multiple seed traits can be utilized in breeding programmes and functional markers are extremely useful in MAS for improvement of those traits and development of rice varieties for various ecosystems.

Abstract 67: Detection and identification of invasive plants and weed seeds using next generation sequencing (NGS)

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To import seeds into Canada, the seed lot must be free of prohibited noxious weeds as weed seeds mixed in with imported soil or crop seeds are one of the main pathways for unintentional introduction of invasive plants into the country. It is also necessary that imported seed lots meet the minimum standards for purity and germination. Therefore, marketplace monitoring samples are taken and tested along with the non-compliant import samples for seed purity and germination. Seed purity testing is done by manually searching the seed sample for contaminating seeds which are then analysed morphologically for species identification. The process can be lengthy due to the number of seeds in the samples but also for samples containing seeds of irregular shapes, small seeds or mixed seeds like wild flower seeds. Furthermore, identification by seed analyst experts can be limited to the genus or family level due to the lack of distinctive morphological characteristics. Next Generation Sequencing (NGS) is an innovative technique allowing simultaneous DNA sequencing of hundreds of thousands of molecules and the tracking of the sequences produced all in one experiment. NGS can be used for metagenomic study which is the detection and identification of species in other species. The species identification is done using sequences of specific common genomic area of the genome namely barcodes. Therefore, a proof of concept project was initiated using the NGS technique to detect and identify weed seeds as well as other crop seeds contaminating imported seed samples using barcode markers.

Abstract 68: Detection of damage in sunflower seeds by wavelet-based spectral descriptors of X-Ray images

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Analysis of seeds is essential for determining seed lot quality and hence sowing value. Although not fully objective, assessing seed quality with radiographic images has been used as alternative to standard laboratory testing. Here, we applied 2-D scale-mixing wavelet transform for automatic processing of radiographic images of sunflower seeds. From the transformed images several spectral indices are derived. These descriptors involve spectral slopes which are directly connected with the degree of image regularity. A methodology paradigm was developed to analyze the images and classify each seed as damaged or undamaged (slight, full). By considering binary and multinomial supervised classification, the rate of correct classification was found to be 82% for damaged and full seeds, and 57% for damaged, slightly damaged, and full seeds.

Abstract 69: Study of biochemical and physiological alterations in seeds *Arabidopsis thaliana* mutants devoid of the 1-Cys Peroxiredoxin (AtPER1)

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Seed development and germination are two critical processes in the life cycle of plants. These processes involve dehydration and rehydration, respectively, which provoke the accumulation of reactive oxygen species (ROS). A high accumulation of ROS may cause oxidative damage to seed cellular constituents, this oxidative stress provokes the delay or even the inhibition of seed germination. Moreover, lower levels of ROS have an important role as secondary messengers. Therefore, oxidants have a dual function in seed physiology (Bailly *et al*., 2008). Among these ROS, the level of hydroperoxides in plants is controlled by a complex network of antioxidants, which include the 1-Cys Peroxiredoxin (Prx), a Cys-based peroxidase localized in the nucleus of seed cells. Arabidopsis thaliana contains a single gene encoding 1-Cys Prx, AtPer1, which is highly expressed during late phases of seed development, yet the function of this nuclear-localized 1-Cys Prx is poorly known. To address this question we have isolated three independent Arabidopsis thaliana lines with T-DNA insertions at the AtPer1 gene. Both RT-PCR and Western blot analyses confirm two mutant lines devoid of AtPer1 transcripts and, consequently of the AtPER1 polypeptide. In silico studies making use of available databases confirmed the high expression of the AtPer1 gene during seed development and initial stages of germination. Furthermore, the promoter of Atper1 contains cis-elements conserved in genes involved in seed maturation and germination. In line with these findings, plate assays indicated slower and poorer germination.

Abstract 70: Biochemical deterioration of corn seed (*Zea mays L.*) var single cross 704 in two storage conditions

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This experiment was conducted to evaluate biochemical seed deterioration of two seed lots of corn that were produced in two locations, had different initial vigor levels and were stored in two different conditions. Flat seeds with two levels of initial vigor (88% and 97%) from two regional corn seed production areas (Karaj and Moghan in Iran) were stored in two storage conditions (controlled store and non-controlled store) for one year and physiological changes were studied. The experiment was set up as factorial based on a completely randomized design with three replications. Results showed that the origin of seed production, initial vigor and seed storage had a significant effect on seed protein content. Comparison of means showed that seeds produced in Karaj had greater protein content than Moghan seeds and seeds with high initial vigor (97%) had accumulated more protein compared to those with low initial vigor. Also when seeds were stored in controlled storage they had more protein than when stored in a non-controlled warehouse. The origin of seed production, initial vigor and seed storage also had a significant effect on seed starch content. Comparison of means showed that seeds produced in Moghan had more starch than seeds produced in Karaj and seeds with high initial vigor and those stored in the controlled warehouse had more starch than seeds with low initial vigor and those stored in the non-controlled warehouse. When seeds deteriorated as a result of inappropriate temperature and moisture conditions, seed components are damaged, resulting in a reduction in vigor. Initial vigor and storage conditions had a significant effect on malondialdehyde seed content. Comparison of means indicated that seeds with high initial vigor and stored in the controlled store, had less deterioration because of adequate storage conditions such as temperature and humidity conditions.

Abstract 71: A new automated seedling imaging system (Vigor-S) for assessment of maize seed vigour

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The development of new computational resources to accurately evaluate the physiological quality of seed lots have shown promising results for quality control programs performed by seed companies. The main objective of this research was to evaluate the physiological quality of maize seeds using a new automated analysis system of seedling images (Vigor-S) in comparison to recommended methods to assess seed vigour, including the Seedling Vigor Imaging System (SVIS®) created by The Ohio State University/USA. Vigor-S is in the final stages of development by research groups of the Department of Crop Science/University of São Paulo and EMBRAPA Agricultural Instruments (CNPDIA). Seedling images were captured by a scanner and processed by computer to automatically generate numerical values representing a vigour index plus an index of uniformity of seedling development, and also data for seedling length (separately or not for shoot and root growth). Two maize hybrids, each represented by seven seed lots, were tested for germination and vigour (cold test, accelerated ageing, and field seedling emergence) as well as the SVIS® and the Vigor-S. In both systems images of 3-day old seedlings after germination at 25°C were evaluated. The Vigor-S was demonstrated to be a consistent alternative to assess maize seed vigour providing similar information to that obtained by accelerated ageing with saturated salt and SVIS®. Vigor-S also revealed high potential for evaluating seed physiological quality of other species.

Abstract 72: Accelerated aging effect on lipid peroxidation and antioxidant enzyme activity of two soybean cultivars

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In order to identify the effects of accelerated aging on enzyme and lipid peroxidation in soybean an experiment was conducted based on a completely randomized design by factorial arrangement with five levels of seed aging (3, 6, 9 and 12 days of aging) and without aging, and two cultivars (Katol and Sahar) with three replications in the seed testing laboratory of the Agricultural and Natural Research Center of Golestan. Accelerated aging was achieved by incubating the seed in a closed plastic box at 40°C and close to 100% relative humidity for up to 12 days. Seed viability, electrical conductivity, lipid peroxidation, superoxide dismutase, catalase, peroxidase, poly phenol oxidase and ascorbate peroxidase activity were measured in aged and non-aged seeds. The results indicated that activity of antioxidant enzymes, with the exception of peroxidase, were reduced and this caused higher malondialdehyde (MDA) and electrolyte leakage (EC). All of these effects caused lower seed vigor during aging. There was significant correlation between EC with percentage of seed germination and this showed that EC could be considered as a rapid method for the evaluation of aged seed. Results showed that Katol cultivar was more tolerant than Sahar cultivar to seed aging.

POSTER SESSION 5 – CONSERVATION AND USE OF GENETIC REOURCES FOR CROP, FOREST AND WILD SPECIES

Abstract 73: Development of tests for seed quality in native seeds used in habitat restoration

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Habitat loss and degradation has led to an increased demand for native seeds for grassland restoration. However, despite increased commercial seed production, there has been little work to establish quality testing of native seeds. There are neither regulatory levels nor industry standards. Most native seed sold is not certified and is traded without germination results being available, since there is a lack of methodology for many native species. Furthermore, the presence and range of different types of dormancy in native populations has to be overcome. A survey was conducted of the seed quality of 12 European native species from 24 EU seed suppliers. In order to achieve this, protocols were developed to determine conditions for dormancy breaking and germination in these species. This survey revealed quality problems in a high proportion of the native seed lots being sold in the EU market; the total germination (% radicle emergence) found between samples of the same species ranged from 0% to 99%. More rapid assessments of germination than in a germination test were achieved following modification of the tetrazolium (TZ) and electrical conductivity (EC) tests for application to native species. TZ staining was highly predictive of germination in 8 species ($r^2 \geq 0.90$), providing rapid identification of low quality lots in just 2 days. The significant correlation between EC and final germination in *Cyanus segetum* suggested a further test of germination that could be completed in 24 hours. The potential for tests to identify low vigour lots will also be discussed.

Abstract 74: Effect of different storage condition on seed viability and germination potential of *Withania somnifera*

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Withania somnifera is commonly known as *Aswagandha*. It belongs to the family Solanaceae. *Withania somnifera* is under intensive utilization because of its wide ranging medicinal potential. Roots and leaves are used in medicine. It contains an essential oil, ipuranal, a crystalline alcohol, whittaniol, alkaloids namely withanine and somniferene. The tuberous roots are astringent, bitter, somniferous, thermogenic, stimulant, diabetic, tonic, etc. So far, this plant has been exploited mostly from its natural habitat. Therefore, there is an immediate need for conservation and mass production through seeds to meet individual needs and market demand. The present study was undertaken to standardize the method of storage of seeds of *Withania somnifera* to enhance the seed longevity and germination potential. Among various methods tried, the

highest germination (58%) was found with seed stored in plastic bottles after 10 months of storage; this was followed by seed storage at 4°C (52.33%) and storage in earthen pots (48%). In comparison control seeds had 39.33% germination after 10 months of storage. Afterwards the germination was found to be declining. After 14 months of storage control seeds did not germinate, but seed stored in plastic bottles had a germination of 10.33%. Similarly the germination velocity index, seedling length and seedling biomass were found to be promising with plastic bottle storage. The effective range of moisture was found to be 6.35 to 6%. The beneficial effect of seed storage in plastic bottles was found to be significant at the 0.05% level.

Abstract 75: Study of the activities of catalase and peroxidase enzymes during development and maturation of hybrid maize seed (*Zea mays* L.), and their relation to acquisition of desiccation tolerance

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Acquisition of desiccation tolerance (ADT) is a very important process that determines seed survival during storage and under conditions of stress. For an accurate evaluation of the duration and timing of the start and completion of ADT in hybrid maize seed, an experiment was designed to detect changes in catalase and peroxidase activity and starch content in different environmental conditions. The investigation was conducted with three replicates and five planting dates at Karaj, Iran. The results show that the hybrid maize seed achieved maximum seed quality (physiological maturity) before the end of seed filling (mass maturity) and black layer formation. ADT began from the preliminary stages of development, and the seeds germinated after drying. Differences in the quality of fresh and dried seeds under different conditions is an indicator of the effect of ADT condition on seed quality. ADT was completed before mass maturity. Comparing this process with changes in catalase and peroxidase activity and starch content confirms the role of catalase activity and starch content in the beginning and completion of ADT. The results show that if desiccation occurs at moderate temperature, ADT is completed earlier, and dried seeds reach their maximum germination level sooner. Mean germination time shows that seed quality deteriorates after completion of ADT. Catalase and peroxidase activity changes had no strong relation with air temperature changes, but were affected by developmental stage and completion stages of the ADT process. This research confirms the role of catalase activity and starch content in beginning and completing ADT, but more research is required to ascertain the role of peroxidase. This research also confirms that environmental conditions during ADT, especially temperature, have an undeniable role in determining seed quality.

Abstract 76: Equilibrium relative humidity and seed testing

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The current ISTA rules include both gravimetric moisture content (mc) determination and moisture meter analysis as methods of seed moisture status determination. An alternative technique not validated by ISTA is equilibrium relative humidity (eRH) or water activity determination. Seeds are hygroscopic; they absorb or desorb water to be in equilibrium with their surroundings. Seed eRH is determined using a hygrometer, which measures the humidity of air surrounding a seed sample. The test is non-destructive and particularly appropriate for samples with small numbers of seeds, or for valuable collections. In some samples not all components have the same mc. However, provided that the sample is in equilibrium with itself, all components will have the same eRH. In some species the embryo will have greater oil content, and lower mc, than the rest of the seed. In very chaffy samples seed mc will be higher than that of non-seed material. This principal is also true for pelleted seeds, the pelleting material can be at a significantly lower mc than that of the seed. In these cases measurement of eRH can be more meaningful than a mc measurement. The Moisture Committee have formed an eRH working group to investigate variables that could influence an eRH result and develop a standardised test method. Initial comparative studies will be carried out with *Triticum*, *Poa* and *Quercus* species, with the aim of ensuring that the test is repeatable and results reproducible across laboratories. We anticipate that this work will lead to the production of a new section or chapter in the ISTA rules.

Abstract 77: Germination conditions for *Sedum takesimense* Nakai

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This study was conducted to determine optimal germination conditions and establish an efficient method for promoting the germination of *Sedum takesimense* Nakai, which is endemic to South Korea. *S. takesimense* seeds demonstrated the highest germination rate under temperature and light conditions of 25°C and 68.4%, respectively. The germination increased by 15.1% when planted in light conditions compared to dark conditions. The results of single and mixed use of plant growth regulators GA_3 and kinetin at various concentrations revealed that kinetin inhibited growth regardless of concentration or whether it was given as a single or mixed treatment, whereas the GA_3 single-treatment groups showed considerable improvement in germination, germination energy, and T50. In particular, the GA_3 200 mgL^{-1} treatment group showed germination rates as high as 94.6%. Additionally, when treated with GA_3 200 mgL^{-1}, the germination rate of *S. takesimense* under dark conditions was higher than that of GA_3 non-treatment light and dark groups by 11.6% and 27.1%, respectively. In terms of the dependence of germination rate on light quality, the red-LED group yielded the highest germination rate (73.0%), whereas the blue-LED group was outperformed by the white light group. Based on these results, it can be concluded that red-LED light and GA_3 200 mgL^{-1} treatment conditions enhance the germination rate of *S. takesimense* Nakai, while red-LED light reduces its underground growth.

Abstract 78: Effect of gibberellic acid (GA_3) on the germination of wild plants native to Korea

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The effects of different concentrations of gibberellic acid (GA_3) on seed germination were determined using 24 species of Korean wild plants. The seeds used in this study were stored for 1–3 years in the Korea Wild Plant Seed Bank (KWPSB) at 4°C and 40% RH. Before pretreatment with GA_3, the seeds were sterilized with 2% NaOCl (two drops per 100 mL) for 8 min and rinsed three times with distilled water. Sterilized seeds were soaked in solutions containing different concentrations of GA_3 (0, 200, 500, and 1000 mgL-1) for 20 h at 4°C in the dark. Seed germination tests were performed on absorbent paper placed in Petri dishes at 20°C in the light. In particular *Parasenecio auriculata* (DC.) H. Koyama, *Barbarea orthoceras* Ledeb., and *Cardamine flexuosa* With. only germinated in the presence of GA_3. In our previous study, *C. flexuosa* showed a germination rate of less than 10% under light and at various temperature conditions (15, 20, 25, and 30°C) or in the dark at 25°C. However, GA_3 treatment increased the germination rate to more than 50%. In this study, GA_3 promotes germination of the 24 species of wild seeds. These results suggest that GA_3 treatment is a simple and useful method for promoting the germination of wild seeds, with the optimal concentration being dependent on the species.

Abstract 79: Korea Wild Plant Seed Bank for the conservation and utilization of plant resources

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With the adoption of the Nagoya Protocol, reinforcement of the sovereign rights of biological resources, and the access regulation and profit of biological resources have all emerged earnestly. Moreover, the sovereignty of each country regarding the biological genetic resources has been recognized with the Nagoya Protocol development, and the competition among countries for biomass, the source material of the biological industry, is expected to become fiercer. Seeds are a unique plant resource, which make restoration and proliferation possible. The country should systematically retrieve and preserve native plants to maintain healthy ecosystems and ensure utilization of source material within the biological industries. Therefore, the National Institute of Biological Resources operates the Korea Wild Plant Seed Bank to ensure the diversity of seeds, industry–academia research, and pursue comprehensive research of resources and customized businesses for consumer growth. To date, 10,000 seeds of approximately 2,000 species of wild plants have been collected, and species-specific studies on germination characteristics for foundation of long-term preservation and mass proliferation are being conducted. Moreover, the NIBR Biomass Rental and Sale System (www.nibr.go.kr/specimen) was opened in July 2014 to enable online search and purchasing of various biological resources, including seeds. The Korea Wild Plant Seed Bank will continuously strive towards: 1) permanently conserving endangered plants and rare plant seed resources in preparation for possible extinction, 2) habitat restoration through wild plant mass proliferations, 3) supplying foundations for biological industry source materials through mass proliferation of useful plant resources, and 4) establishing a stable supply of biomass for domestic wild plant restoration and supply.

Abstract 80: Efforts towards conserving the critically endangered *Protea roupelliae* subsp. *hamiltonii* population in South Africa

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Plant conservation is imperative and due to the genetic material housed within a seed, their storage plays a key role in that process. Successful seed storage is determined by many conditions such as seed water content (wc), humidity and storage temperature; with the former two aspects influencing the effect storage temperature might have on the seed. In the assessment of storage regimes pre-and post-storage germination tests are required to determine the effect of storage conditions on the viability and vigour of the seeds. *Protea roupelliae* Meisn subsp. *hamiltonii* Beard ex Rourke (Proteaceae) is an indigenous, serotinous shrub with the last remaining population of less than 1000 individuals being located at the Dr. Hamilton reserve, Barberton, South Africa. The seeds which exhibit considerable variation in water content (3.5% - 9.8%) and mass (11mg - 28mg) were germinated in Petri dishes between sheets of filter paper under growth room conditions (25°C and 14h photoperiod) for 60 days. Fresh seeds with a mass <18mg showed no germination whilst seeds of mass ≥18mg showed 98% germination. As the species is serotinous, it was also possible to distinguish one, two and three year old seeds on the parent plant. One year old seeds had the highest cumulative germination of 86% compared with two (56%) and three (58%) year old seeds [$F(14) = 4.538$, $P = 0.034$]. The one year old seeds also had a higher germination index, followed by the three year old seeds. The seed water content of *Protea roupelliae* subsp. *hamiltonii* is variable both within and between years with a coefficient of variation (CV) of 20.80, 19.18 and 18.77 respectively. Variation in water content (wc) is known to have an effect on the storage behaviour of seeds. It was therefore necessary to reduce the variation in water content prior to any storage tests. Seeds were equilibrated over silica gel and a saturated solution of calcium nitrate ($Ca(NO_3)_2$) with a relative humidity of 50% in a closed desiccator at 25°C for 5 days and 40 days respectively. Seeds held over silica gel did not reach equilibrium whereas those held over $Ca(NO_3)_2$, reached equilibrium at 8%, 7.4% and 7.9% respectively and the variation decreased. Seeds equilibrated over calcium nitrate were then stored at 25, 4, -20 and -70°C respectively, for six and twelve months. Post six months storage analysis showed that the equilibrated seed water content had been retained (with little variation) and this was particularly true for the three year old seeds stored at -70 and -20°C. Germination was also the highest for seed stored at those temperatures (76% and 75%). The 12 month experiment is ongoing.

Abstract 81: Comparison of different top-down strategies for measurement uncertainty estimation in quantification of genetically modified organisms

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In order to comply with the regulation about the adventitious presence of genetically modified organisms (GMOs), GMO quantification has become a critical issue. Therefore, different methods were applied to evaluate the measurement uncertainty for GMO quantitative analysis, and then to characterize the dispersion of the quantity values which can be reasonably ascribed to the measurand. In this study we took two GM powdered certified reference material (CRM) as unknown sample and quantitative analyzed by real-time polymerase chain reaction (real-time PCR). Three top-down approaches - intermediate precision, repeatability standard deviation, and Del Gaudio *et al*. (2012) approach - evaluated the uncertainty of GM crop quantitative analysis. Based on the results of the measurement uncertainty estimation, a conformity assessment was performed. Results showed that intermediate precision and repeatability standard deviation featured consistent measurement uncertainty for the same events; variation sources were primarily derived from sample preparation processes. Del Gaudio *et al*. (2012) approach could confirm that whether the quantitative analysis method was up to the standard in the laboratory.

Abstract 82: Changes in maize seed quality after five years of storage

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Proper storage conditions are essential to maintain high viability of seeds. The aim of the study was to determine the effect of storage environment on 10 Polish varieties of maize cultivated in one location. After determining germination ability seed material was placed in two types of storage conditions: variable storage conditions (ambient temperature and humidity) and long term storage (constant temperature 0°C and low humidity). During 5 years of storage seeds of both treatments were tested for germination ability and vigour measured by the cold-test and radicle emergence test (since the year 2013). The effect of storage conditions depends on the variety of maize. After five years at ambient storage conditions germination ranged from 32 – 94 %, mean 69%; results of the cold test for most of the samples had reached zero, except for one variety, Narew 32%. The mean result for the radicle emergence test was 65%, the result for variety Narew was 84% and was one of the highest. In long term storage conditions mean germination was maintained at 98% (range 93 – 99%). Reaction of seeds to the cold test varied from 20 to 71%, mean 42%. In the radicle emergence most of the samples achieved 100%. Storage of maize for five years in ambient conditions caused a decrease in seed quality.

Abstract 83: Effects of hydration-dehydration treatments on the quality of seeds after twelve years of storage

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Seed invigoration through hydration-dehydration treatments is a widely used technique to improve seed germination performance. These methods, commonly known as “priming”, involve a partial hydration of the seeds and a subsequent drying. Seed hydration is maintained at a level where metabolic processes can take place but radicle protrusion is prevented. Seed priming has been often associated with a decrease in the longevity of stored seeds. However, many studies have demonstrated that these procedures may also improve the storability of the seeds by reversing the effects of seed ageing. In this work, seed samples of oat, barley and lentil were subjected to different hydro-priming treatments and, after twelve years of storage, their germinability and vigour were evaluated. The treatments applied were: T0, control without priming; T1, seed imbibition on water-saturated filter paper for 24 hours; T2 and T3, seed humidification in a water saturated atmosphere for three and six days, respectively. After priming the seeds were desiccated to 5.0 ± 1 % seed moisture content and stored in air-tight containers at room temperature and at −18°C (genebank conditions). Seed imbibition for 24 hours (T1) had a negative effect on seed quality that was noticeable immediately after treatment in most of the samples. On the contrary, the 3-days humidification (T2) significantly increased the seed germinability or vigour after twelve years of storage. T3 promoted seed fungi proliferation and was in general detrimental for seed quality and longevity. Hydro-priming is a simple and economic treatment that, in some cases, might contribute to extend the lifespan of seeds stored in genebanks.

Abstract 84: Occurrence of seed-borne fungi in rye (*Secale cereale*) germplasm after thirty years of storage in the National Spanish genebank. Relation with seed viability.

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The National Center for Plant Genetic Resources of Spain (CRF-INIA) maintains more than 45,000 seed accessions under medium term (active collection) or long term (base collections) storage conditions. The samples are stored at 5-7% seed moisture content and temperatures of -4°C and -18°C for the active and base collection, respectively. The rye collection includes around 500 accessions and many of them have showed important decreases in seed viability during the storage period, due to the short life-span of these seeds. Seed-borne pathogens may have negative effects on seed quality and may pose a risk of spreading diseases through material exchange. The environmental conditions used in genebanks to increase seed longevity usually favor the survival of seed-borne mycoflora. The initial diversity of seed fungi is closely

related to the specific environmental conditions during the year of collection but may vary over time due to differences in longevity among microorganisms. Limited studies have been conducted on the effects of seed-borne fungi in the longevity of seeds during long-term storage. In this work, seed fungi analyses were carried out on 20 rye samples stored for more than 30 years in the CRF active collection. Twenty four different fungi species were identified and *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* were the most frequently isolated fungal genus. Correlations between seed infection and seed germination percentages were determined and isolates of the most represented fungal species were inoculated in fresh rye seeds to evaluate their effects on seed germination. Given the obtained results, the possible influence of seed fungi on the longevity of seeds stored under genebank conditions is discussed.

Abstract 85: The provocation test is the best method to determine pre-harvest sprouting resistance in triticale

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In comparison to its parents, wheat and rye, triticale (x Triticosecale Wittm.) has a lower production and yield growth rate (FAOSTAT, 2013). Wet weather conditions during ripening and harvest cause yield and quality losses by pre-harvest sprouting in many triticale genotypes. Successful breeding towards sprouting resistance requires a reliable test method and a suitable selection tool. In a three year project we tested the following methods for their correlation with sprouting resistance observed in fields: “provocation test” (i.e. irrigation of spikes in the glasshouse for several days and assessing the visible degree of sprouting), artificial irrigation in field plots, falling number, α-amylase-activity, separate near-infrared-spectroscopy measurements of glumes and seeds and germination tests under various conditions. Each test method was carried out with spikes or seeds of at least 36 different triticale lines harvested from field experiments grown in 3 years and 5 locations in Germany. The provocation test showed the best correlations to sprouting occurrence in the field. The artificial irrigation achieved a similar potential, but this method is very space and labour intensive. Correlations between field sprouting occurrence/“provocation test” and falling number, α-amylase-activity, separate near-infrared-spectroscopy measurements of glumes and seeds and germination tests under various conditions were all insufficient for recommending these tests for decision making in breeding programs.

Abstract 86: Effects of the environment on seed quality of *Amburana cearensis* under different storage. A species from the Caatinga biome

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Seed storage is important to maintain maximum physiological and physical quality and seed health, to reduce ageing (deterioration), and to preserve seed for use in the future. During storage seeds deteriorate, an irreversible process which involves physiological, biochemical and physical changes. *A. cearensis* is an endangered species and ex situ seed storage may be fundamental to prevent its extinction. In this research seeds were subjected to different storage environments: airtight containers in a refrigerator; airtight containers in a laboratory environment; laboratory environment without airtight containers and cryopreservation for 24 months. Every three months seeds were removed from storage to monitor viability through germination tests and to check the total soluble sugar and reducing sugar content. Our results indicate that the environment affected seed physiology. Total sugar content and the reducing sugar levels of *A. cearensis* were highly variable across all the environments analysed. Large variations in the content of these sugars were noted between different times of storage and some associations with seed storage physiology were evident. T50 values were greater with storage in liquid nitrogen. Germination was slower (higher T50 values) and reduced when storage was carried out in a laboratory environment without airtight containers.

Abstract 87: Flow cytometry as a tool for assessing viability of long-term stored seeds

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The aim of this study is to establish a fast and accurate screening method for determining the viability of seeds stored in genebanks using flow cytometry. After mitotic division cells undergo the first growth phase (G1), then a phase of DNA synthesis and a second phase of growth (G2). The nuclei of diploid cells in the G1 phase contain 2C DNA and those in the G2 phase contain 4C DNA. Flow cytometry allows determination of the quantitative ratio between cells which are in different stages of the cell cycle (G2/G1), which provides information on the physiology of seed, its stage of development, maturity, and advancement of germination. G2/G1 ratio was considered to be a marker of germination, used to track the processes of seed conditioning. Experiments were carried out with rye kernels subjected to different methods and time of storage which resulted in different levels of germination of these kernels. The results show that in non-germinating seeds the G2/G1 ratio is higher. Thus, the question is: Is there a possibility to establish a threshold value of G2/G1 which would divide germinating and non-germinating seeds?

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